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MISCELLANEOUS PUBLICATION 20

IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

III. STUDIES OF FUNGI, METAZOA, PROTOZOA, AND RICKETTSIAE

Warren R. Sanborn

MARCH 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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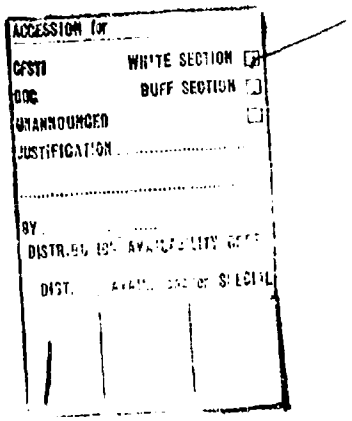

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Warren R. Sanborn

March 1968

Technical Information Division
AEROBIOLOGY AND EVALUATION LABORATORY

FOREWORD

The use of immunofluorescence, or fluorescent antibody, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The volume of literature related to immunofluorescence or fluorescent antibody and covering use of this technique has expanded explosively over the relatively few years since its inception. This expanding literature volume bears witness to the basic value of the technique. Through 1954, only about 40 articles had been published. In the next two years, 58 were added. During 1957 and 1958 there were 83 and 96, respectively. By 1961 the annual figure had reached more than 260 articles. For this supplementary second edition, the figures for 1963, 1964, and 1965 are 551, 764, and 678, respectively. These totals are testimony to Dr. Coons' genius.

Although it would be virtually impossible to cite every article that refers to the use of immunofluorescence, an attempt has been made to approach that limit. To that end, more than 445 journals were searched. In addition, six abstracting journals and the computer system of the National Library of Medicine, MEDLARS, were employed. Fifteen languages are represented. Translations were provided by colleagues of the compiler, government translating services, abstractors, and the compiler. The earliest entry in the original edition was 1905. In the present edition, entries covering the years 1963, 1964, and 1965 are the primary ones included, but there are also a few earlier entries not listed in the first edition. Further entries for 1966 and 1967 are now being compiled; these will be incorporated into further revisions of this bibliography.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. This entire second edition, Miscellaneous Publication 20, has the same overall title, "Immunofluorescence, an Annotated Bibliography," as the first edition (Miscellaneous Publication 3). The present edition also has the same six-volume structure: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending

undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author. A seventh volume, "Author and Subject Indexes," has been added to further aid the investigator in his search for articles relevant to his interest area.

Abstracts for citations in this edition have been prepared or modified in keeping with the central theme, the application of immunofluorescence to various problems. If the primary emphasis in the original article was immunofluorescence and the author's summary reflected this, the summary was generally left unchanged, except for minor changes and abbreviations simply to save space. In other instances, it was necessary to write a new abstract in order to indicate the proper place of immunofluorescent technique in the study. At the same time, the main point of such articles was maintained in abbreviated form in the abstract. Hopefully, this approach will be successful in bringing the application of immunofluorescence to the attention of the reader, while preserving each author's ideas at the same time. It is further hoped that this bibliography will aid investigators in avoiding duplication of effort and thus contribute to even greater and more imaginative applications of immunofluorescence.

Accession numbers have been assigned consecutively to citations throughout all six volumes of this edition. The plan for further future volumes allows this simple system. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in each volume.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

To avoid excess duplication and unwieldy size, the second parts of Volumes V and VI contain only basic citations for articles printed in the other four volumes. However, titles of articles are included to assist the reader in selection of those citations of possible interest. As in the other volumes, the references are placed in subject categories and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous forms. For unmistakable identification, they are listed below.

BSA	bovine serum albumin
DANS	a. 1-dimethylaminonaphthalene-5-sulfonic acid b. 5-dimethylamino-1-naphthalene sulfonic acid or chloride form.

FA	fluorescent antibody
FIC	fluorescein isocyanate
FITC	fluorescein isothiocyanate
FTA	fluorescent treponemal antibody
FTA abs	fluorescent treponemal antibody absorbed
FTA-200	a modification of the above based on serum dilution
PAP	primary atypical pneumonia
PAS	para-aminosalicylic acid
PBS	phosphate-buffered saline
RB 200	a. lissamine rhodamine RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B
TPFA	Treponema pallidum fluorescent antibody
TPI	Treponema pallidum immobilization

Generally, the citations follow the format prescribed by the second edition of Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler began to collect this immunofluorescence literature in 1957 while he was stationed at U.S. Navy Preventive Medicine Unit No. 2, Norfolk, Virginia. The literature collection became more intense and organized after 1959 when he was transferred to Fort Detrick, Frederick, Maryland. Following his further transfer to the Microbiology Department of the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he continued this work with the encouragement and support of both of these latter installations. Work on the second edition began in 1964, and it has continued through support from both the U.S. Army and the Bureau of Medicine and Surgery of the U.S. Navy. This volume was completed while the compiler was assigned to U.S. Navy Medical Research Unit No. 3, FPO New York, 09527, where he is currently serving as head of the Bacteriology Department.

The information in these volumes was originally recorded on coded marginal punch cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701, for improvement of the subsequent editions. Reader assistance in this area will be deeply appreciated.

ACKNOWLEDGMENTS

The essential team effort required for development of this immunofluorescence bibliography cannot be overstressed. As with many projects of this nature, the talents, advice, guidance, and assistance of many people led to the completion of this second edition. The compiler is deeply grateful to the many people who have contributed.

Financial support for this project at first was absorbed by the Pathology Division and the Walter Reed Army Medical Unit, Fort Detrick. However, completion of the first edition (through 1962) was made possible by special financial assistance from Physical Defense Division, Fort Detrick, under Dr. Charles R. Phillips. I am extremely grateful to him for his aid. Expenses for this second edition were primarily met through a generous grant from U.S. Navy Bureau of Medicine and Surgery, Preventive Medicine Division, under CAPT J. Millar, MC, USN. Many administration expenses also were borne by the Naval Medical Research Institute and by Fort Detrick.

A number of libraries kindly donated their services. In spite of the unusual requests required by this project, these libraries were very helpful and willingly assisted, often providing valuable suggestions. Libraries primarily involved were the Technical Library, Fort Detrick, under Mr. Charles N. Bebee and later Miss Joyce A. Wolfe, and the Technical Reference Library, Naval Medical Research Institute, Mrs. T.P. Robinson, librarian. Much valuable as sistance also was rendered by the National Institutes of Health Library, Miss R. Connelly, reference librarian, the National Library of Medicine, and the library of the Walter Reed Army Medical Unit, Fort Detrick. The staff members of these libraries were both helpful and patient. Without such fine assistance, the work could not have been completed.

It is a pleasure to acknowledge the highly competent secretarial help. Secretaries providing their capable and untiring talents were: Miss Sandra Rosenblatt, Miss Linda L. Zimmerman, Mrs. Marguerite M. Matovich, Mrs. Gene Heaven, Mrs. Linda Franklin, Mrs. Alberta Brown, Mrs. Margaret Raheb, and a number of others. Valuable assistance in double-checking problem references was provided by Mrs. Catherine F. Eaves and Mrs. Mary J. Gretzinger. Dr. George H. Nelson was a willing consultant for classification problems. Dr. Harold W. Batchelor provided an essential key to the development of this work by introducing the compiler to marginal punch card systems and guiding him in their application.

The Technical Information Division, under Mr. Gerald W. Beveridge, continually provided all types of assistance in addition to a home base from which to work. My gratitude for this cannot be fully expressed.

Last, but by no means least, the essential editorial work receives my highest praise. The tireless efforts, patience, and driving force supplied by these people were the prime factors in bringing this edition to completion. Mrs. Madeline Warnock Harp, in charge, Mrs. Mary D. Nelson, and Mrs. Ruth P. Zmudzinski all spent many hard weeks of work on this project. I shall always be indebted to them.

ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume III contains 208 annotated literature citations, arranged according to major subject areas, and a complete author index.

CONTENTS

I. FUNGI

A. DEEP MYCOSES

5932

Brandsberg, J.W.; Tosh, F.E.; Furcolow, M.L. 1964. Concurrent infection with Histoplasma capsulatum and Blastomyces dermatitidis. New Engl. J. Med. 270:874-877.

Five cases of simultaneous pulmonary infections of human beings with Histoplasma capsulatum and Blastomyces dermatitidis are reported. The possibility of the existence of a hybrid resulting from fusion of B. dermatitidis and H. capsulatum is discussed, and the evidence supporting such a contention is presented. FA provided some evidence. The role of such a hybrid, if it exists, is considered a possible explanation for some atypical isolates of B. dermatitidis and H. capsulatum.

5933

Cerottini, J.C.; Jaton, J.; Froidevaux, T.; Isliker, H. 1964. Immunofluorescence by means of fragments of antibodies. Helv. Physiol. Pharmacol. Acta 22:C9-C12. In French.

The gamma globulin fraction of rabbit serum immunized against Histoplasma capsulatum was separated into three parts by means of papain digestion and chromatography on a carboxymethyl-cellulose column. FITC was added to the fractions, and the fractions added to the antigen as outlined. Fraction I and II always are fixed on the antigen giving a positive fluorescence. Fraction I from a non-immune animal does not show fluorescence. Fraction III does not react whether the animal is immune or not. It might be better to use Fractions I or II instead of the total gamma globulin to avoid unspecific fluorescence. BA-46-12110.

5934

Converse, J.L.; Castleberry, M.W.; Snyder, E.M. 1963. Experimental viable vaccine against pulmonary coccidioidomycosis in monkeys. J. Bacteriol. 86:1041-1051.

Monkeys vaccinated by subcutaneous injection in the forearm with 10 to 100 million viable Coccidioides immitis arthrospores were protected against respiratory challenge with approximately 7,000 viable arthrospores administered 6 months after vaccination. Protection was evident from: the healthy appearance throughout 4 months after respiratory challenge; negative chest X-rays at 15, 30, 60, and 120 days; and only very minor histopathological pulmonary changes on autopsy at 120 days, with negative lung cultures in 80 per cent of the animals. This was in striking contrast to the outward clinical appearance of

control monkeys that were unvaccinated or had received nonviable arthrospore vaccines. These monkeys showed severe disease, positive X-rays, massive pulmonary destruction, positive lung cultures, and death of five of nine animals. The appearance of a very few spherules accompanied by very minor pathological changes in the lungs of some of the dissemination controls (subcutaneous viable vaccination without respiratory challenge) indicated possible dissemination from the primary cutaneous infection. Oral transmission from the cutaneous lesions could not be ruled out. FA was used as a check of fungus in tissues.

5935

Converse, J.L.; Pakes, S.P.; Snyder, E.M.; Castleberry, M.W. 1964. Experimental primary cutaneous coccidioidomycosis in the monkey. J. Bacteriol. 87:81-85.

Primary cutaneous coccidioidomycosis was studied in the monkey to find a suitable strain of Coccidioides immitis for use as a viable vaccine. Intradermal inoculation produced more severe vaccination reactions than did subcutaneous injection. A subcutaneous vaccine dose of ten arthrospores resulted in less reaction than a 100-spore dose. Dissemination beyond the regional lymph nodes did not occur after injection of ten spores of even the most virulent strains of C. immitis. Two of the five strains tested exhibited very mild vaccination reactions and appeared to have been cleared from the tissues upon necropsy at 10 months postvaccination. These two strains, Cash and CW1, appear promising for further immunological studies with a viable vaccine. Lung sections were checked by FA.

5936

Kaplan, W.; Clifford, M.K. 1964. Production of fluorescent antibody reagents specific for the tissue form of Coccidioides immitis. Amer. Rev. Resp. Dis. 89:651-658.

Five different lots of rabbit Coccidioides immitis antiglobulins were tagged with fluorescein isothiocyanate. These reagents brightly stained endospores and contents of spherules formed in vivo. The labeled antibodies also cross-reacted with Histoplasma capsulatum, Blastomyces dermatitidis, and other heterologous fungi. Absorption of these conjugates with yeast cells of H. capsulatum eliminated all nonspecific activity. Two of the absorbed reagents reacted strongly with the tissue forms of C. immitis. These conjugates had been prepared from globulins produced by rabbits infected with the fungus and by rabbits immunized with killed arthrospores. By dilution, the former conjugate was also rendered specific for tissue forms of C. immitis. The specific conjugates were successfully used to detect C. immitis in clinical materials from confirmed cases of coccidioidomycosis.

5937

Kaplan, W.; Kaufman, L. 1963. Specific fluorescent antiglobulins for the detection and identification of Blastomyces dermatitidis yeast-phase cells. Mycopathol. Mycol. Appl. 19:173-180.

Two lots of rabbit anti-Blastomyces dermatitidis globulins were conjugated with fluorescein isothiocyanate. These reagents brightly stained elements of the yeast and mycelial phases of ten strains of B. dermatitidis. In addition, the labeled antibodies cross-reacted with elements of the yeast and mycelial phases of seven strains of Histoplasma capsulatum and cells of numerous other heterologous fungi. Adsorption of one lot of labeled antibodies twice with yeast cells of H. capsulatum and once with elements of Geotrichum candidum rendered the conjugate specific for the yeast phase of B. dermatitidis. Three adsorptions with yeast cells of H. capsulatum followed by a single adsorption with elements of G. candidum rendered the second conjugate specific for yeast-phase cells of B. dermatitidis. The specific reagents did not react with the mycelial phase of this fungus.

5938

Kaplan, W.; Kaufman, L.; Clifford, M.K. 1963. Production of a fluorescent antibody reagent specific for the tissue form of Coccidioides immitis. Bacteriol. Proc. M140:89.

A definitive diagnosis of coccidioidomycosis by conventional mycological techniques requires demonstration of the causal agent, Coccidioides immitis, in clinical specimens or its isolation. C. immitis fluorescent antibody reagent specific for the tissue form of this fungus would be of great value for rapid and reliable diagnosis of this disease. C. immitis antisera were produced in rabbits by intravenous inoculation of viable arthrospores. The pooled antisera had complement-fixation titers of 1:2048 with coccidioidin, 1:64 with histoplasmin, 1:8 with Histoplasma capsulatum yeast-phase cells, and 1:32 with Blastomyces dermatitidis yeast-phase cells. Globulin isothiocyanate intensely stained the tissue form and mycelial elements of C. immitis. This conjugate, however, cross-stained yeast and mycelial elements of H. capsulatum, B. dermatitidis, and the cells of eight other heterologous species representing seven genera. Absorption of the conjugate twice with yeast cells of H. capsulatum eliminated all cross-reactivity. The absorbed reagent intensely stained elements of the tissue form of C. immitis. In contrast, the specific conjugate did not react with the mycelial form of this fungus.

5939

Kaufman, L.; Blumer, S. 1965. Occurrence of serotypes among Histoplasma capsulatum strains. Bacteriol. Proc. M10:41.

Antisera were produced in rabbits against four strains of H. capsulatum. Fluorescein isothiocyanate - labeled globulin fractions of the four antisera showed extensive cross-staining with both the homologous and the heterologous strains of H. capsulatum and also with three yeast-phase strains of H. duboisii, indicating that at least one antigen was common to the fungi studied. Homologous and heterologous adsorptions were employed to clarify the antigenic relationship among the H. capsulatum strains. To date, 15 strains of H. capsulatum and 11 strains of H. duboisii have been studied. Our results suggest that at least two and possibly three antigenic serotypes occur in the species H. capsulatum. One is related to H. duboisii.

5940

Kaufman, L.; Brandt, B. 1964. Fluorescent antibody studies of the mycelial form of Histoplasma capsulatum and morphologically similar fungi. J. Bacteriol. 87:120-126.

Fluorescent antibody reagents were developed to differentiate the mycelial form of the pathogen, Histoplasma capsulatum, from several morphologically similar but saprophytic species of the genera Sepedonium and Chrysosporium. Cross-staining and adsorption procedures revealed antigenic relationships among certain species of these three genera and other fungi. The significance of these observations to the diagnostician and taxonomist is discussed.

5941

Kaufman, L.; Brandt, B.; McLaughlin, D. 1964. Evaluation of the fluorescent antibody and agar gel precipitin tests for detecting Histoplasma antibodies in anticomplementary sera. Amer. J. Hyg. 79:181-185.

The agar gel precipitin and fluorescent antibody inhibition tests were used to analyze qualitatively 127 anticomplementary (AC) sera from human beings and dogs for antibodies to Histoplasma capsulatum. Where possible, these results were compared with those obtained by complement-fixation (CF) tests on corresponding repeat non-AC sera. The results of the agar gel and FA inhibition test showed 97 per cent over-all agreement with those obtained by the CF tests. The agar gel and FA inhibition tests proved valuable in screening AC sera. FA also rapidly supplied physicians with presumptive evidence for histoplasmosis.

5942

Kaufman, L.; Kaplan, W. 1963. Serological characterization of pathogenic fungi by means of fluorescent antibodies: I. Antigenic relationships between yeast and mycelial forms of Histoplasma capsulatum and Blastomyces dermatitidis. J. Bacteriol. 85:986-991.

Four antiglobulins prepared against the yeast and mycelial forms of Histoplasma capsulatum and Blastomyces dermatitidis were labeled with fluorescein

isothiocyanate. Cross-staining and adsorption techniques made possible the discernment of five antigenic factors in the fungi studied. The yeast-phase cells of H. capsulatum and B. dermatitidis contained the respective distinct antigens B and D and a common factor E. In addition, the yeast-phase cells of these two species shared antigen C with the mycelial elements of H. capsulatum. The mycelial cells of H. capsulatum and B. dermatitidis shared antigen A with the yeast-phase cells of these two organisms and contained no distinct antigens.

5943

Kaufman, L.; Kaplan, W.; Brandt, B. 1963. Fluorescent antibody studies of Histoplasma capsulatum and related fungi. Bacteriol. Proc. M139:89.

Prerequisite to the recognition of the mycelial form of Histoplasma capsulatum by fluorescent antibody staining is the development of an FA reagent capable of differentiating the pathogen from the morphologically similar species of Sepedonium and Chrysosporium. In an attempt to produce such a reagent, antibodies against the mycelial forms of H. capsulatum, C. histoplasmoides, and S. chrysospermum were produced in rabbits. The reactivity of the three antisera with yeast- and mycelial-phase antigens of H. capsulatum were demonstrated by the complement-fixation test. Homologous as well as heterologous antigen-antibody reactions were characterized by the Ouchterlony method. FA studies with fluorescein-labeled antiglobulins indicated that all three conjugates intensely cross-stained the heterologous fungi. Attempts to render the labeled antiglobulins specific for the homologous fungi were carried out by absorption with the heterologous antigens. This procedure produced reagents capable of differentiating the three genera.

5944

Pine, L.; Kaufman, L.; Boone, C.J. 1964. Comparative fluorescent antibody staining of Histoplasma capsulatum and Histoplasma duboisii with a specific anti - yeast phase H. capsulatum conjugate. Mycopathol. Mycol. Appl. 24:315-326.

The specific anti - yeast phase Histoplasma capsulatum conjugate has been tested against 13 yeast-phase strains of H. capsulatum and nine of H. duboisii. The conjugate was specific for H. capsulatum; no yeast-phase form of H. duboisii obtained in vitro or in vivo reacted with it. The taxonomic implications of these results are discussed.

5945

Porter, B.M.; Comfort, B.K.; Menges, R.W.; Habermann, R.T.; Smith, C.D. 1965. Correlation of fluorescent antibody, histopathology, and culture on tissues from 372 animals examined for histoplasmosis and blastomycosis. J. Bacteriol. 89:748-751.

In a survey in the midwestern United States, tissues from 372 animals of 16 species were examined for Histoplasma capsulatum and Blastomyces dermatitidis. All of the specimens were cultured and studied by the FA technique, and histopathological studies of tissue sections were

done. Among the 372 animals, 300 were negative by all three methods of examination, 34 had blastomycosis, and 21 had histoplasmosis. The remaining 17 were positive for histoplasmosis by the FA technique but were negative by histopathology and culture. An animal was considered to have histoplasmosis or blastomycosis only when the specific fungus was isolated or demonstrated in tissue sections or exudates from cutaneous lesions. Of the 34 blastomycosis cases, 74 per cent were positive by FA, 94 per cent by histopathology, and 32 per cent by culture. Of the 21 histoplasmosis cases, 71 per cent were positive by FA, 76 per cent by histopathology, and 67 per cent by culture. It appeared that FA, histopathology, or culture alone was insufficient for a diagnosis. All three techniques should be employed for the most satisfactory diagnostic results.

5946

Porter, B.M.; Thomas, B.K.; Furcolow, M.L.; Varga, D.T. 1963. Comparison of two Histoplasma capsulatum fluorescent antibody conjugates based on 800 clinical specimens. Bacteriol. Proc. M141:90.

On the basis of 800 clinical specimens, two differently prepared conjugates were compared. In both cases, antiserum prepared against the yeast phase of Histoplasma capsulatum was tagged with fluorescein isothiocyanate. In one case, the conjugate was absorbed three times with tissue powders, once with monkey liver powder and Candida sp. yeast powder, and twice with mouse liver powder. In the second case, the conjugate was absorbed with packed, formalin-killed yeast cells of Blastomyces dermatitidis according to procedures adapted from those outlined by Kaplan and Kaufman in 1961. The conjugate absorbed with packed B. dermatitidis is more specific, requires fewer reagents, and can be diluted for use with clinical specimens.

5947

Porter, B.M.; Thomas, B.K.; Minges, F.W.; Habermann, R.T.; Silby, L.A. 1963. Correlation of fluorescent antibody, histopathology, and culture on tissues from 355 animals examined for histoplasmosis or blastomycosis. Bacteriol. Proc. M143:90.

In a survey in the midwestern United States, tissues from 355 animals of 16 different species were examined for Histoplasma capsulatum and Blastomyces dermatitidis. All specimens were studied by fluorescent antibody, histopathology, and culture. Of the 355 animals, 300 were negative by all three diagnostic methods, 21 were proved histoplasmosis cases, and 34 were proved blastomycosis cases. A case was considered proved if the organism was demonstrated by either culture or histopathology. Of the 21 proved cases of histoplasmosis, 15 were positive by FA, 16 were positive by histopathology, and 14 were positive by culture. Of the 34 proved blastomycosis cases, 25 were positive by FA, 32 were positive by histopathology, and 11 were positive by culture. It appears that neither FA, histopathology, nor culture alone is sufficient for diagnosis. All three techniques should probably be employed for satisfactory diagnostic results.

5948

Silva, M.E.; Kaplan, W. 1965. Specific fluorescein-labeled antiglobulins for the yeast form of Paracoccidioides brasiliensis. Amer. J. Trop. Med. Hyg. 14:290-294.

Two lots of rabbit Paracoccidioides brasiliensis antiglobulins, produced against the yeast form of this fungus, were conjugated with fluorescein isothiocyanate. Both reagents brightly stained elements of the yeast and mycelial forms of 15 isolates of P. brasiliensis. In addition, they cross-reacted with the tissue form of Histoplasma capsulatum, Blastomyces dermatitidis, Sporotrichum schenckii; once, with cells of the mycelial form of Coccidioides immitis, they rendered this conjugate specific for the yeast form of P. brasiliensis. Adsorption of the second conjugate once with mycelial growth of C. immitis followed by a single adsorption with yeast cells of H. capsulatum and one adsorption with cells of Rhodotorula sp. rendered it specific for the yeast form of P. brasiliensis. The specific reagents stained P. brasiliensis cells in smears of clinical materials from four spontaneous human cases of South American blastomycosis. The specific reagents did not react with elements of the mycelial form of P. brasiliensis.

5949

Sotgiu, G.; Mazzoni, A.; Mantovani, A.; Ajello, L.; Palmer, J. 1965. Histoplasma capsulatum: Occurrence in soil from the Emilia-Romagna region of Italy. Science 147:624.

The existence of an area in Europe in which histoplasmosis is endemic was revealed by the isolation of Histoplasma capsulatum from soil. The soil specimen was collected in a chicken yard on a farm near Bologna, Italy. The Emilia-Romagna region had been selected for study because several, apparently autochthonous, human cases of histoplasmosis had originated there. The identity of the fungus was further verified by means of FA whereby the yeast cells induced in vitro were brightly stained with a conjugate specific for the yeast phase of H. capsulatum.

5950

Suter, L.S.; Ulrich, E.W.; Ridgell, J.O. 1965. Antibody in histoplasmosis determined by the indirect fluorescent technique. Bacteriol. Proc. M11:41.

The standard indirect method using fluorescein-conjugated antihuman globulin produced in rabbits was used to titer sera from histoplasmosis patients. Sera from 46 different patients with culturally proved histoplasmosis were studied; most of these were of the chronic pulmonary type. To determine cross-immunity reactions, sera from 19 patients with other systemic fungus diseases were studied. A group of sera from 59 supposedly normal controls was also studied; serum dilutions

of 1:10 to 1:160 were used. In the 46 histoplasmosis patients, the following titers were observed: 1:10 dilution, 4.3 per cent; 1:20, 10.9 per cent; 1:40, 30.4 per cent; 1:80, 19.6 per cent; 1:160, 34.8 per cent. In the 59 normal controls, the following titers were observed: negative in the 1:10 dilution, 62.7 per cent; positive in the following dilutions: 1:10, 16.9 per cent; 1:20, 10.2 per cent; 1:40, 6.8 per cent; 1:80, 1.7 per cent; 1:160, 1.7 per cent. With the few patients studied with other systemic fungus diseases, there was some cross-immunity in blastomycosis.

5951

Thomas, B.K.; Porter, B.M.; Varga, D.T.; Furcolow, M.L.; Brandsberg, J.W. 1963. Correlation of fluorescent antibody and culture on 1489 sputa examined for Histoplasma capsulatum. Bacteriol. Proc. M142:90.

On the basis of 1489 clinical sputum specimens, results were compared between fluorescent antibody and culture in detecting the yeast phase of Histoplasma capsulatum. The antisera prepared against the yeast phase of this organism in rabbits were tagged with fluorescein isothiocyanate. In this study 180 sputa from 44 patients were positive by FA or culture. Eighty-four per cent of the specimens positive by culture were also positive by FA. Two per cent false positives were encountered, the majority coming from treated patients, previously positive. The 24 specimens that were FA-negative and culture-positive were from 11 patients, 8 of whom had been FA-positive on other specimens. Discussion includes improved methods now available that yield greater correlation with culture results.

5952

Yamaguchi, B.T., Jr.; Adriano, S.; Braunstein, H. 1963. Histoplasma capsulatum in the pulmonary primary complex: Immunohistochemical demonstration. Amer. J. Pathol. 43:713-719.

Paraffin blocks of 24 inactive pulmonary primary complexes contained yeast-like structures resembling H. capsulatum. It was possible to identify these organisms as H. capsulatum in 22 cases by fluorescent antibody technique. Five of the 22 had been previously cultured for fungi, with negative results. There appears little doubt that the structures observed did indeed represent H. capsulatum. Successful immunohistochemical identification of nonviable fungi in conventionally prepared sections suggests the employment of this technique in instances where culture has been either unsuccessful or omitted.

B. YEASTS

5953

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1965. Cell wall replication in Saccharomyces cerevisiae. Bacteriol. Proc. G146:38.

The bud formation in Saccharomyces cerevisiae was studied by differential labeling of living cells with fluorescent and nonfluorescent antibody. Examination of smears at consecutive intervals of 15 minutes showed that the bud was nonfluorescent, but the mother yeast remained as discrete fluorescent areas. The bud formation was initiated as a small bulge that enlarged and gradually developed into a small bud. Further increase in size of the bud was accompanied by the formation of a cross wall and constriction at the base area to separate the bud from the mother yeast. It appears that the cell wall of the bud was newly synthesized first at the base area, and then added to the actively growing new bud in a direction away from the base. After the separation of the yeast cells, the birth and bud scars were clearly visible on the fluorescent cell wall.

5954

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1965. Cell wall replication in Saccharomyces cerevisiae. Can. J. Microbiol. 11:953-957.

Cell wall growth and bud formation in Saccharomyces cerevisiae were studied by FA. Labeled cells were grown in a glucose yeast extract broth and examined at 15-minute intervals. The new cell wall was largely non-fluorescent; the old wall showed no reduction of fluorescence during growth of the bud. Bud formation was initiated as a small bulge on the cell wall, and further increase in size was accompanied by the formation of a constriction around the basal end that led to the separation of the bud from the mother yeast cell. The actively growing area of the bud was an annular band close to the base. It appears that the cell wall of the bud was, almost entirely, newly synthesized and contained very little old cell wall material. The process of wall synthesis is compared with the pattern found in several bacteria and with what is known of the process in other fungi.

5955

Goos, R.D.; Summers, D.F. 1964. Use of fluorescent antibody techniques in observations on the morphogenesis of fungi. Mycologia 56:701-707.

Observations on cells of Candida albicans stained by FA indicate that wall material of the parent cell is incorporated into the wall of daughter cells or into hyphal walls when these are produced. A diminution in fluorescent intensity along the length of the newly formed hyphae was apparent, suggesting a dilution of parent wall material with continued

growth. Fluorescence of newly formed elements was observed when the cells were grown in nonlabeled immune serum ruling out the possibility that these cells became stained by possibly dissociated antibodies. Conidia of Fusarium oxysporum f. cubense stained intensely, but newly emerged germ tubes failed to do so. Sites of germ tube emergence were readily apparent as nonfluorescing areas of the conidium wall. When young colonies were treated with FITC labeled serum, the phialide tips, the microconidia, and macroconidia all stained intensely with antisera prepared against microconidia, although hyphal walls failed to react with the stain, suggesting an antigenic dissimilarity in the walls of the hyphae and conidia.

5956

Hasenclever, H.F. 1965. The antigens of Candida albicans. Amer. Rev. Resp. Dis. 92(Suppl.):150-158.

The antigen studies reported were mainly performed by agglutination tests, but FA studies using absorbed sera also confirmed the findings for group specificity.

5957

Jacobson, A. 1963. Cryptococcosis: On the rise. Illinois Med. J. 123:258-260.

Indirect FA may be used to demonstrate antibody to Cryptococcus. It is specific and apparently the only satisfactory test available.

5958

Kaufman, L.; Blumer, S. 1964. Flucrescent antibody staining and agglutination reactions of Cryptococcus neoformans antibodies. Bacteriol. Proc. M158:73.

The unequivocal identification of C. neoformans by conventional biochemical and cultural procedures requires not less than 1 week, the time needed to differentiate C. neoformans from similar fungi such as the Candida and Torulopsis species and saprophytic cryptococci. Agglutination reactions of pooled rabbit C. neoformans antiserum showing a homologous agglutinin titer of 1:160 were compared with the agglutination and FA-staining reactions of FITC-labeled antiglobulin derived from the same serum. The conjugate had a protein concentration of 2.0 grams per 100 ml and a homologous agglutinin titer of 1:80. Studies of 29 strains of C. neoformans, various species of Cryptococcus and Candida, and one strain of T. glabrata, using antiserum and labeled antiglobulin, gave positive results in agglutination and FA tests with antigens from all the homologous species and with some of the heterologous antigens. Specific agglutinins for C. neoformans were obtained by absorbing either the antiserum or conjugate with these three organisms: C. neoformans var. uniguttulatus, Candida albicans, and C. curvata. Although the absorbed conjugate consistently agglutinated

all the C. neoformans strains studied, it failed to stain some of them. Conversely, positive FA-staining reactions were not always correlated with positive agglutination reactions. C. neoformans can be rapidly and accurately identified by use of specific agglutinins but not by FA staining.

5959

Kent, S.P. 1963. A study of mucins in tissue sections by the fluorescent antibody technique: III. The specificity of antibody to salivary gland mucins and the effect of chemical alterations of mucins on the specificity of the antibody. Ann. N.Y. Acad. Sci. 106:389-401.

Cow submaxillary mucin, human submaxillary mucin, the capsule of Cryptococcus, and blood group substances A and H were observed by the fluorescent antibody technique in formalin-fixed, paraffin-embedded tissue sections. The effect of a variety of chemical alterations of these antigens on their reactions with specific antibody was studied. The following are suggested: Free carboxyl and hydroxyl groups are essential to the reaction of the above antigens with specific antibody. Cow submaxillary mucin contains sialic acid, which is essential to the reaction of this mucin with its specific antibody; blood-group-specific substance A either does not contain sialic acid or if sialic acid is present it is not necessary for the serologic reaction; the introduction of phenylhydrazine or p-hydrazenobenzoic acid into the antigens following periodic acid oxidation often restores the reaction of the antigen with its specific antibody, but it is associated with a number of cross-reactions not seen in the controls.

5960

Lehner, T. 1965. Immunofluorescent investigation of Candida albicans antibodies in human saliva. Arch. Oral Biol. 10:975-980.

Parallel quantitative estimation of antibodies to Candida albicans Group A in serum and saliva was carried out in controls, carriers, and infected subjects, using indirect FA. Antibodies were demonstrated in serum and saliva. Controls gave titers in serum up to 1:8, and in saliva up to X64 concentrated saliva. Carriers showed levels in serum up to 1:16, and up to X16 in concentrated saliva. Candidiasis patients had serum titers up to 1:512, and salivary titers up to 1:8. Thus, the three groups differed quantitatively in their immunological status. A serum titer of 1:32 or above, and a saliva titer of 1:1 or above therefore indicates clinical infection, and so aids in the differential diagnosis of candidiasis.

5961

Leone, R. 1964. Cutaneous candidiasis: I. Immunofluorescence, agglutination, and fungistatic reaction. Minerva Dermatol. 39:231-235. In Italian.

The immunofluorescence technique was used to study the serological behavior of subjects suffering from candidiasis and from noncandidic dermatoses. In healthy subjects used as controls, the possible correlation between antibodies and the presence of saprophytic Candida at the cutaneous surface was also studied. Fungistatic power in candidid and noncandidid dermatosis was also studied. The immunofluorescence technique does not lead to results substantially different from agglutination, with which it runs parallel in the majority of cases. The research for fungistatic power is usually negative in cutaneous candidiasis.

5962

Mizuno, S.; Yudate, H. 1963. Rapid diagnostic method for fungi by means of fluorescent antibody technique. Rinsho Byori 11:391-399. In Japanese.

For the FA technique used as a serological procedure in diagnosis of fungi, preparation of antigens from Candida, Saccharomyces, and Torulopsis varieties, using rabbits, preparation of specific immune sera, and preparation of fluorescent-labeled antibodies are described. C. albicans, C. stellatoidea, C. guilliermondii, and T. glabata were diagnosed specifically by the FA method. From vaginal smear samples, C. albicans was detected in 65 of 96 cases; T. glabata in 21 of 96; C. stellatoidea and C. guilliermondii in none. Ten of 96 were undetectable. These results compared favorably with those obtained by other methods. Good results in identification of C. albicans were also obtained from smear samples from newborn infants. Methods of eliminating nonspecific fluorescence are suggested.

5963

Schamschula, R.G. 1964. The application of the fluorescent antibody technique to the detection of Candida albicans in oral pathological material. Australian J. Exp. Biol. Med. Sci. 42:173-180.

The fluorescent antibody technique was applied to the detection of Candida albicans in oral smears. C. albicans was identified by cultural methods in 44 of 50 cases of suspected candidiasis. When the fluorescent antibody technique was used, the organism was detected and identified in the original pathological material in 95.4 per cent of the cases. This technique appears to be superior to other commonly used microscopical methods as far as the facility of detection of the organism is concerned and has the added advantage of serological specificity.

5964

Weyman-Rzucidlo, D. 1965. A histochemical and immunological study of Candida albicans chlamydospores: II. Immunofluorescent study of the antigenic properties of C. albicans chlamydospores. Acta Microbiol. Pol. 14:247-256.

A distinct specific antigen was found in capsular parts of the chlamydospores of Candida albicans by indirect FA. Results of the present study are discussed in connection with the previous histochemical investigations on the structure morphogenesis of C. albicans chlamydospores.

5965

Yudate, H. 1963. Application of the fluorescent antibody technique in obstetrics and gynecology with emphasis on fundamental and clinical studies in immunological and mycological diagnosis. J. Jap. Obstet. Gynecol. Soc. 15:131-140. In Japanese.

Application of FA for rapid diagnosis of infectious diseases in the fields of gynecology and obstetrics, was made to improve differential diagnosis of fungi on smears from clinical and experimental sources. With four antisera produced against Candida albicans, C. stellatoidea, C. guilliermondii, and Torulopsis glablata, specific identification of these four fungi was possible. No cross-reaction was observed with absorbed antiserum. With absorbed antisera, an attempt was made to identify these four fungi in vaginal smears and smears obtained from oral swabs of newborn. C. albicans and T. glablata were detected but not C. stellatoidea and C. guilliermondii. The rate of detection by direct swab of the lesion, cultivation in Bouillon medium for a short period, and by centrifugation was improved by these methods in this order. Among FA, biological, and serological methods, biological and FA technique gave equally good results. The identification of these four fungi was possible even when mixed with other fungi. Rabbit antiserum could be produced easily using heated antigens. The method could also be applied to study of the spread of C. albicans in experimentally induced infections and to the detection of antigen in impression smears of organs and in frozen tissue sections.

C. OTHER FUNGI AND RELATED STUDIES

5966

Al-Doory, Y.; Gordon, M.A. 1963. Application of fluorescent antibody procedures to the study of pathogenic dematiaceous fungi: I. Differentiation of Cladosporium carrionii and Cladosporium bantianum. J. Bacteriol. 86:332-338.

The well-known polymorphism and confusing similarity of cultures of pathogenic dematiaceous molds and other so-called black yeasts make individual species difficult to identify and even more perplexing to classify. Serological investigations of antigen relationships have

proved of value in previous attempts to resolve these taxonomic problems, but thus far no fluorescent antibody studies in this field have been reported. In the present investigations, fluorescein-labeled antisera to both Cladosporium carrionii and C. bantianum reacted with a number of dematiaceous species in addition to the homologous organisms, but selective dilution and absorption resulted in conjugates specific for each of these two species. Staining was confined to the periphery of both hyphae and spores, and age was a factor in the affinity of these structures for fluorescent antibody. There is evidence that antigen diffuses centrifugally from the hyphae during growth on agar media.

5967

Al-Doory, Y.; Gordon, M.A. 1963. Further fluorescent antibody studies of pathogenic dematiaceous fungi. Bacteriol. Proc. M144:90.

Fluorescein-conjugated antiglobulins to five pathogenic species of dematiaceous fungi of the Cladosporium-Fonsecaea group were prepared and tested against homologous and heterologous antigens. The specificity of these conjugates and their application to accurate identification of the respective species have been investigated by testing with 75 pathogenic and 25 nonpathogenic strains of dematiaceous fungi plus several nondematiaceous species. Direct and indirect staining methods were compared. Two methods of antibody absorption, one of them representing a new technique, were applied to eliminate the numerous cross-reactions occurring among these fungi. Cladosporium carrionii and C. bantianum, which are very similar morphologically in culture, were separated readily by FA.

5968

Beno, D.W.; Allen, O.N. 1964. Immuno-fluorescent staining for the identification of Puccinia sorghi germinated urediospores. Phytopathology 54:872-873.

FA staining was accomplished by first fixing spores on slides with acetone or ethanol, then overlaying with FITC conjugated with immune rabbit globulin. After suitable washing with buffered saline, air drying, and adding phosphate-buffered saline and glycerol to retard evaporation and improve fluorescence, the mounts were observed for fluorescence. This provides a rapid method of identifying unknown lots of P. sorghi urediospores. BA-46-27101.

5969

Chick, E.W. 1965. Fluorescence microscopy and other special procedures for diagnosis and study of mycotic infections. Amer. Rev. Resp. Dis. 92:175-179.

Many of the problems in the study of immunogenic and immunologic properties of fungi relate to the lack of specific antigen preparations that would be highly sensitive but without cross-reactive activity. To this end a multitude of various fractionation studies have been carried out. The

results have seldom met the expectations. Current serologic tests indicate that a variety of different antibodies may exist. Such antibodies are defined by their activity in a given test situation using certain types of antigen preparations, and the relationship between these antibodies is not always clear. Careful histochemical studies and the use of varied fluorescence microscopy techniques may provide information about antigens that have not been possibly altered or distorted by a harvesting procedure. The use of these methods has already produced valuable information about antigen sites and antigen composition. Further developments in techniques and their more widespread use could play a prominent role in the advance of antigenic studies of fungi.

5970

Cole, R.M. 1965. Symposium on the fine structure and replication of bacteria and their parts: III. Bacterial cell wall replication followed by immunofluorescence. Bacteriol. Rev. 29:326-344.

This is an interpretive and critical report. The author urges further application of FA to the study of surface-antigen replication in walled microorganisms. Confirmation or denial of controversial points in this study area will follow only from such further study. FA has clear advantages over any other method for cell wall study. The chief advantage is the ability to apply a specific label to the wall of a living cell.

5971

Gonzalez-Ochoa, A.; Felix, D.; Anaya, M. 1964. Immunofluorescence in sporotrichosis: Comparative study of the different diagnostic procedures. Rev. Mex. Lab. Clin. 16:2:45-47. In Spanish.

FA is compared to the cultural and the sporotricine skin test methods in 12 cases suspected of sporotrichosis. In seven the fungus was isolated and FA detected Sporotrichum schenckii. The skin test was positive also. In four cases in which another etiology was demonstrated, the three methods were negative. In one doubtful case with positive skin test, the culture and FA were negative. BA-47-79041.

5972

Gonzalez-Ochoa, A.; Kaplan, W. 1964. The use of fluorescent antibodies in the study of some infectious diseases: II. Fast diagnosis of sporotrichosis. Gac. Med. Mex. 94:309-313. In Spanish.

The clinical types and forms of the Sporotrichum schenckii infection are described. The results of the FA technique are reported in exudate smears of 34 cases of sporotrichosis, one of pulmonary localization, and others tegumentary sporotrichosis. Of 27 cases in which the culture of the pathogen showed S. schenckii, FA allowed diagnosis of the fungus

in 24 (89 per cent). One of the cases with megative culture was positive to FA, but the sporotricine cutaneous test was also positive. The advantage of FA when compared to the culture is the short time it takes. BA-46-95156.

5973

Gordon, M.A.; Al-Doory, Y. 1965. Application of fluorescent antibody procedures to the study of pathogenic dematiaceous fungi: II. Serological relationships of the genus Fonsecaea. J. Bacteriol. 89:551-556.

The staining spectra of fluorescein-labeled antiglobulins to Fonsecaea compactum, F. dermatitidis, and F. pedrosoi were determined with respect to 71 strains of morphologically related and 21 miscellaneous molds, including so-called black yeasts. All three conjugates showed considerable reaction with saprophytic Cladosporium spp. F. dermatitidis appeared to be most closely related serologically to the genus Cladosporium; F. compactum conjugate was fairly specific, and the few nonspecific reactions of diluted F. pedrosoi reagent were almost without exception with species of Fonsecaea and Cladosporium. Most strains of F. pedrosoi were unstained by F. compactum conjugate, and there was very little relationship shown between either of these species and Phialophora verrucosa. Conjugates of two strains of P. verrucosa failed to react with any of the three species of Fonsecaea. The possible significance of these results for classification of the black yeasts and other dematiaceous molds is discussed.

5974

Hermanek, P. 1965. The inclusion of a limited number of fungus species into the circle of differential diagnosis. Wiener Klin. Wochensch. 77:116-118. In German.

Identification of fungi from morphology in tissue is very difficult. Problems are presence of atypical tissue forms, presence of unexpected hyphal forms in tissues, and influence of change from germination- to hyphal-form in tissue. All tests of a histological fungus determination proceed only from the hypothesis that there is, in the circle of differential diagnosis, only a limited number of fungus species. This is untenable. In biopsies, one part of the material must be kept for microbiological culture. FA may be useful for identification.

5975

Kaplan, W.; Gonzalez-Ochoa, A.G. 1963. Application of the fluorescent antibody technique to the rapid diagnosis of sporotrichosis. J. Lab. Clin. Med. 62:835-841.

Smears of lesion exudates from 34 patients suspected of having sporotrichosis were examined by the direct fluorescent antibody technique and the results were compared with those obtained by culture. Sporotrichum schenckii cells were found by the fluorescent antibody technique in smears from

24, or 89 per cent, of 27 culturally positive individuals. One of seven culturally negative individuals was also found to be positive by the fluorescent antibody procedure. This one individual responded to potassium iodide therapy. These results indicate that the direct FA technique can be employed as a rapid screening procedure for sporotrichosis.

5976

Kumar, D.; Patton, R.F. 1964. Fluorescent antibody technique for detection of Polyporus tomentosus. Phytopathology 54:898.

Presence of Polyporus tomentosus in mixed cultures, soil, and infected roots of pine was detected by FA. When root sections and soil samples were stained with P. tomentosus antiserum labeled with FITC and examined under a fluorescence microscope, characteristic fluorescence was detected only at the site of homologous fungus. Hyphae of heterologous fungi and surrounding soil particles or root tissue showed no cross-reaction. Specificity of stain was confirmed on smears from a blended mixture of fungus cultures, including P. tomentosus. Only P. tomentosus mycelium was stained while the rest revealed characteristic grayish autofluorescence. The reaction was successfully blocked. No staining occurred with normal conjugate. High antibody titer, purity of gamma globulins before conjugation, and optimum F:P ratio were critical factors in achieving specificity of the reaction. The results suggest the possibility of the preparation of a specific FA stain for any fungus and that its use can be helpful in studying the distribution pattern of fungi that are difficult to isolate from natural sources. Complete article.

5977

Miura, T.; Kasai, T. 1964. Autofluorescence of pathogenic fungi. Tohoku J. Exp. Med. 82:158-163.

Autofluorescence of pathogenic fungi was investigated. Smears were made from the surface of the growth on 4 per cent glucose agar and were fixed by heating, absolute alcohol, or formalin and were placed under the fluorescence microscope. The fungi tested showed various kinds of autofluorescence. There were considerable differences in brightness or in color tone of autofluorescence due to the difference of species, but none due to difference of strains. Of all fungal species tested, Microsporum japonicum and Epidermophyton floccosum gave the most brilliant autofluorescence; its color tone was pale blue. Fixation methods affected the brightness of autofluorescence, and heating was most effective in allowing the most brilliant autofluorescence.

5978

Miura, T.; Kasai, T. 1964. Difference in result of the fluorescent antibody staining of dermatophytes due to the difference of fixing procedure. Tohoku J. Exp. Med. 84:72-80.

The probable relationship between the intensity of the fluorescent antibody staining reaction of dermatophytes and fixing procedure of the smear was investigated. Smears of dermatophytes, including Trichophyton rubrum, T. interdigitale, T. asteroides, Epidermophyton floccosum, and Microsporum japonicum, were fixed by heating, by ethanol, or by formalin, or were left to be air-dried without fixation. They were stained with either anti - T. rubrum or anti - T. interdigitale fluorescent antibody. The experiments have led to the conclusion that, of the fixing techniques employed, heating was the most desirable. Smears fixed by heating could hardly be stained with any of the conjugates, and they gave the most brilliant autofluorescence.

5979

Pier, A.C.; Richard, J.; Farrell, E. 1964. Fluorescent antibody and cultural techniques in cutaneous streptothricosis. Amer. J. Vet. Res. 25:1014-1020.

The application of fluorescent antibody techniques proved helpful in detecting Dermatophilus congolensis in exudate suspensions. Antigenic similarity of four strains of D. congolensis was proposed on the basis of absorption experiments resulting in diminution in the intensity of specific fluorescence of culture smears. Filtration of ground exudate suspensions through 1.2-mu pore size filters facilitated the isolation of D. congolensis.

5980

Ryschenkow, E.; Wertlake, P.T. 1965. Visualization of fungi and other microorganisms on Millipore filter by fluorescent antibody technique. Bacteriol. Proc. M23:43.

A technique has been developed applicable to fungi and other microorganisms in fluid that yields microorganisms with specific fluorescence that contrasts crisply with a dark Millipore filter background. Both direct and indirect fluorescent methods were successful. For the direct method, three volumes of homologous fluorescent antisera were added to one volume of washed saline suspension of organisms, mixed, and allowed to stand for 30 minutes at 24 C. The mixture was then washed two times with buffered saline, resuspended in saline, and passed through a 0.45-micron Millipore filter with a Swinny hypodermic adapter. The filter was dried at 37 C for 30 minutes and placed on a slide with immersion oil and cover slip for examination. The indirect method was similar except that the suspension was originally exposed to untagged homologous antisera, and fluorescent

antiglobulin was added to the washed, sedimented cells. These procedures are especially recommended for rapid identification of small numbers of microorganisms in body fluids that are normally sterile, or in early or scant broth cultures when it is important to prevent loss of cells.

5981

Schmidt, E.L.; Bankole, R.O. 1965. Specificity of immunofluorescent staining for study of Aspergillus flavus in soil. Appl. Microbiol. 13:673-679.

Fluorescein-labeled antiserum prepared with Aspergillus flavus strain CS was tested for specificity by staining fungi grown in soil in the vicinity of buried slides. All 14 strains of A. flavus fluoresced as intensely or nearly as intensely as the antigen control. Among 21 isolates of species of Aspergillus other than A. flavus, 17 reacted with moderate to low fluorescence at intensities readily distinguishable from that of A. flavus. The fluorescence of the remaining four cultures was indistinguishable from that of A. flavus. Fungi other than aspergilli were generally nonreactive. Interfering cross-reactions were encountered for one strain of Spicaria and one strain of Stemphylium. Three isolates could not be evaluated because of interfering autofluorescence. Twenty-two isolates were either wholly negative or displayed little fluorescence. Agglutination tests between each of the fungi and A. flavus CS serum revealed close agreement between agglutination titer and fluorescent-staining reaction. Unknown fungi freshly isolated from soil were FA stained. Only one isolate was positive. That one proved to be A. flavus. In a simplified ecological model, the FA technique was used to follow the development of A. flavus in mixed culture in soil.

5982

Takeuchi, Ikuo. 1963. Immunochemical and immunohistochemical studies on the development of the cellular slime mold Dictyostelium mucoroides. Develop. Biol. 8:1-26.

Hyperimmune sera were produced in rabbits against the spores of D. mucoroides. The heated antisera agglutinated spores as well as vegetative and interphase amebas, with no significant differences in titers. Precipitin ring tests of the antisera with extracts of cells at various stages of development indicated a marked increase in reactivity at the migrating pseudoplasmodium stages and a further increase at the spore stage. In order to study localization of antigens and combining groups at various stages of development, immunohistochemical studies were made by using fluorescein-conjugated antispore sera, both absorbed and unabsorbed. The staining of the cell surfaces indicated that there is a change in the surface in the interphase, this taking place some time after the amebas have finished feeding. Thereafter the cell surface remains unchanged until the time of spore formation. Antibody-stained cytoplasmic granules

were observed at all stages except the vegetative. During interphase, cells begin to synthesize a new combining group in or on certain granules in the cytoplasm. Location of differentiation and variation was studied. The significance of the cell sorting in the normal development of the cellular slime molds was discussed and compared with the development of animal embryos.

5983

Tonomura, K.; Tanabe, O. 1964. Localization of cell-bound alpha amylase in Aspergillus oryzae demonstrated by fluorescent antibody technique. J. Bacteriol. 87:226-227.

Alpha amylase may be located on cell surfaces.

II. METAZOA

A. FLATWORMS

5984

Cookson, L.O.C. 1963. Some investigations with the fluorescent antibody technique: II. Experiments with advanced fluorescent equipment. Cent. Afr. J. Med. 9:469-478.

These experiments confirmed previous findings in schistosome diagnosis. The microscopy equipment used is described in detail. Both the direct and the indirect FA tests proved to be sound tools for diagnosis of schistosomiasis. FA antiglobulin testing is probably the most sensitive and specific schistosomiasis test available. Disadvantages include inabilility to distinguish between species of schistosomes. Cross-antigenicity with other flukes such as Fasciola gigantica and F. hepatica was also demonstrated. Cercariae were the antigen of choice for the test.

5985

Foster, W.B.; Hanson, W.L. 1965. Antibodies in mice infected with Hymenolepis microstoma against freshly excysted cysticercoids as demonstrated by the fluorescent antibody technique. J. Parasitol. 51(Suppl.):62.

Hymenolepis microstoma, a tapeworm that parasitizes the lumen of the bile duct of certain rodents, causes gross enlargement of the bile duct of the albino mouse as well as damage to other organs including the liver. Test antisera were obtained from female albino mice that were infected orally with five to ten cysticercoids of H. microstoma. Recently excysted cysticercoids of H. microstoma were used as test antigen. Cysticercoids were obtained from Tribolium confusum, excysted by treatment with 1 per cent pepsin (pH 2.0) for 15 minutes followed by a mixture of 0.15 per cent bile and 0.10 per cent trypsin (pH 8.0), and the excysted worms were washed several times in Hanks BSS to remove all enzymes. The indirect FA test gave positive results when compared with control sera. Pooled sera of mice infected with H. microstoma for 20 to 25 days produced titers as high as 1:320 when tested against cysticercoids with FA. The time of appearance of detectable antibodies was observed to be as early as 11 days in one mouse and 12 days in one other. Complete article.

5986

Francisco, B.F.; Azelia, P.P. 1964. Presence of antigens in calcareous corpuscles of cysticercus. Rev. Inst. Med. Trop. Sao Paulo 6:114-116.

Immunofluorescence studies have been performed with histological sections of Cysticercus cellulosae and anticysticercus immune serum. Positive

reactions revealed antigens from this parasite playing a role in the immune response of natural infections located in calcareous corpuscles of cysticercus. There are no previous records on this fact and it might be the key to obtaining more specific antigenic extracts of some helminths.

5987

Pellegrino, J. 1963. Serologic diagnosis of schistosomiasis mansoni: I. Comparative study of complement fixation, cercarial flocculation and immunofluorescence tests. Rev. Inst. Med. Trop. Sao Paulo 5:147-153.

The tests were performed in serum samples from 307 patients with active schistosomiasis mansoni and the results were as follows: complement fixation test: 96.1 per cent positive, 0.3 per cent doubtful, and 3.6 per cent negative; flocculation test: 92.2 per cent positive, 2.9 per cent doubtful, and 4.9 per cent negative; FA test: 85.7 per cent positive, 5.2 per cent doubtful, and 9.1 per cent negative. The CF test was negative in all controls. Flocculation and immunofluorescence tests were positive in 5 per cent and doubtful in 4 per cent of the control sera. CF was the most sensitive and specific test for the diagnosis of schistosomiasis mansoni and must be considered as the method of choice for the diagnosis of individual cases. For epidemiological surveys, the reliability for each serological test was analyzed and discussed.

5988

Ramirez-De Pita, V.; Velez-Pratt, G.; Biagi, F. 1965. Immunofluorescence detectable antigens in calcareous corpuscles of Cysticercus cellulosae. Rev. Fac. Med. Univ. Nac. Autonoma. Mex. 7:379-383. In Spanish.

A procedure for obtaining tissue-free calcareous corpuscles of Cysticercus cellulosae is described. The antigenic protein material appears in the homogenized fluid. It is absent from the NaOH and HCl treated corpuscles. The homogenized fluid gives gel precipitation bands with serum from rabbits inoculated with Cysticercus cellulosae antigen. BA-47-38435.

5989

Thorpe, E. 1965. An immunocytochemical study with Fasciola hepatica. Parasitology 55:209-214.

Results are described of a study of the distribution of the antigens in F. hepatica at various stages of development, and in the liver and spleen of experimentally infected rats, using globulins labeled with fluorescein isothiocyanate from rats and sheep infected with F. hepatica. Possible uses of this technique in aiding the diagnosis of fascioliasis are indicated.

5990

Yokogawa, M. 1964. Immunological study of parasites. Naika Hol an 13:92-98. In Japanese.

The criteria for intradermal and complement fixation tests for lung fluke disease, schistosomiasis, and other parasitic diseases are discussed. Practical value of the precipitin test is small. In immunobiological diagnoses, parasitic larvae of a certain age are used as antigens and diagnosis is made by observing the changes in the larvae. Among the tests studied are the Sarles phenomenon, miracidial immobilization, circumoval precipitin test, cercarial Huellen reaction, cercarial agglutination test, and the FA test. FA is convenient for collective testing.

B. NEMATODES

5991

Baar, H.S.; Galindo, J. 1965. Ossifying pulmonary granulomatosis due to larvae of ascaris. J. Clin. Pathol. 18:737-742.

A unique case of ossifying pulmonary granulomata is described in which the presence of larvae of Ascaris lumbricoides within the granulomata was demonstrated by means of FA.

5992

Baratawidjaja, R.K.; Hewson, A.; Labzoffsky, N.A. 1963. Fluorescent antibody staining in the serodiagnosis of trichinosis. Can. J. Microbiol. 9:625-628.

A fluorescent staining procedure for Trichinella spiralis and the appearance of the stained larvae are described. The applicability of the method to the serodiagnosis of trichinosis was investigated. The results obtained with both the experimental and the human sera agreed well with the complement-fixation results. In titrating nine experimental sera and 36 sera from parasitologically proved or clinically diagnosed cases of trichinosis in humans, higher titers were obtained by FA, indicating that this test is somewhat more sensitive.

5993

Bird, A.F. 1964. Serological studies on the plant parasitic nematode, Meloidogyne javanica. Exp. Parasitol. 15:350-360.

Experiments are described showing that injection of living larvae of the plant parasitic root-knot nematode, Meloidogyne javanica, into rabbits results in the production of antibody. The antibody was shown, by gel filtration and ultracentrifugal analysis, to be located primarily in

the gamma globulin fraction. Starch-gel electrophoresis of antiserum and globulin-fraction-containing antibody demonstrated that an alpha-2 globulin was also involved. By using FA it was shown that the antibody reacts with antigenic material exuded from the excretory pore and to a lesser extent, from the buccal stylet of both larvae and adults. The gelatinous matrix exuded from the adult female also appeared to have antigenic properties as, to a lesser extent, did the cuticle. As the larvae aged, fewer produced precipitates in antiserum.

5994

Calderon, S. 1964. Immunological studies on onchocerciasis. Salud Publica Mex. 6:553-559. In Spanish.

In accord with other reports from the literature, the author stresses the low antigenicity of onchocercal extracts for the rabbit. With the use of Freund's complete adjuvant, however, an immune serum with a precipitating titer of 1:64 was obtained and employed as positive control in the search for antibodies in the serum of 38 onchocercous individuals. Trials using the capillary and agar precipitation, Boyden's technique, immunoadherence, or immunofluorescence (Lucasse) yielded negative results. The latter test, however, gave nonspecific positive fluorescence of microfilariae analogous to that seen with sera from patients with rheumatoid arthritis. An interpretation of the above results is offered.

5995

Crandall, C.A.; Echevarria, R.; Arean, V.M. 1963. Localization of antibody binding sites in the larvae of Ascaris lumbricoides var. suum by means of fluorescent technics. Exp. Parasitol. 14:296-303.

Antibody binding sites in Ascaris lumbricoides var. suum were determined by direct and indirect fluorescent antibody techniques. Binding sites on embryonated eggs, freshly hatched second-stage larvae, and larvae isolated from and within the lungs and livers of experimentally infected mice, as well as sections of adult worms, were investigated. Specific fluorescent staining was observed in oral, anal, cuticular, and excretory pore precipitates of larvae isolated from tissues and in the cuticle of whole and sectioned larvae. No precipitates were observed on second-stage larvae; however, the cuticle stained specifically in both whole and sectioned second-stage larvae when incubated in immune fluorescent globulin. Similarly, specific staining of the larval cuticle was noted in sections of embryonated eggs. The fertilization membrane of the latter stained nonspecifically. No staining of the cuticle in sections of adult worms was observed. Only the periphery of the muscle cells showed specific staining.

5996

Crandall, C.A.; Echevarria, R.; Arean, V.M. 1963. Localization of antibody-binding sites in Ascaris suum by means of fluorescent techniques. J. Parasitol. 49(Suppl):33.

Embryonated eggs, second-stage larvae, and larvae isolated from the livers and lungs of experimentally infected animals were incubated in fluorescein-labeled antiglobulin. Controls consisted of incubation and staining with fluorescein-labeled normal globulin. Rhodamine bovine albumin was added to the fluorescent globulins in some cases to decrease nonspecific staining. Specific staining was observed in oral, anal, excretory pore, and cuticular precipitates of larvae isolated from tissues. No precipitates were formed on second-stage larvae incubated in immune globulins. The cuticle of whole and sectioned second- and third-stage larvae stained specifically. Specific fluorescent staining of the larval cuticle was observed in sectioned embryonated eggs whereas the 'fertilization' membrane stained nonspecifically. No staining of the cuticle of sectioned adult worms resulted with either immune or normal fluorescent globulin. Complete article.

5997

Labzoffsky, N.A.; Baratawidjaja, R.K.; Kuitunen, E.; Lewis, F.N.; Kavelman, D.A.; Morrissey, L.P. 1964. Immunofluorescence as an aid in the early diagnosis of trichinosis. Can. Med. Ass. J. 90:920-921.

The established serological tests for trichinosis are often negative during the period when laboratory investigation is most likely to be useful. Another serological test, the immunofluorescence test, appears to be more promising in this respect on the basis of studies involving experimental animals and human patients. In two rabbits orally infected with Trichinella spiralis larvae, antibodies were demonstrable by immunofluorescence on the 4th day after infection, by complement fixation on the 8th and 10th days, and by the precipitin test on the 13th and 28th days. In three human cases the immunofluorescence antibody test was positive 2 weeks after onset when the earliest blood samples were available. Precipitin and complement-fixation tests did not become positive until the end of the 4th week. The immunofluorescence test thus becomes positive at least 2 weeks earlier than the others, a factor that undoubtedly increases its value in diagnosis.

5998

Lucasse, C.; Hoeppli, R. 1963. Immunofluorescence in onchocerciasis. Z. Tropenmed. Parasitol. 14:262-269.

Immunofluorescence was studied on the sera of 50 persons infected with Onchocerca volvulus over a long period to detect a possible correlation between antibody level and age of patients, number of microfilariae, number of nodules, and skin alterations. We found that the age of the

patients and the number of microfilariae in the skin are not related to the strength of the antibody response. On the other hand, in our material a relationship was found between the number of nodules accompanied by skin changes and the antibody reaction. The majority of patients with very marked skin alterations and many nodules showed a comparatively low antibody titer. The greatest number of very strong and strong reactions was found in patients with a moderate number of nodules and moderate skin changes, therefore younger infections.

5999

Mitchell, J.R. 1964. Detection of Toxacara canis antibodies with the fluorescent antibody technique. Proc. Soc. Exp. Biol. Med. 117:267-270.

The indirect fluorescent antibody technique was used to detect antibody against Toxacara canis in four groups of sera. In a group of animals infected with T. canis or Ascaris suum, T. canis antibodies appeared within 2 weeks of infection and a weak reaction with anti-Ascaris sera appeared after repeated infection. Sera from a group of rabbits and monkeys inoculated or infected with various bacterial, viral, and parasitic antigens gave no significant reaction. Five of 25 children, age 19 to 22 months, and 17 of 49 normal adult blood donors gave a significant reaction.

6000

Nayak, D.P. 1965. Epizootiology of swine influenza. Diss. Abstr. 26:1292.

The complex etiology of swine infuenza involves a pneumotropic virus and Haemophilus influenzae suis. The object of this investigation was (1) to determine the progressive pathogenesis of swine influenza virus (S-15) in its experimental and natural host by FA and histopathological technique; (2) to evaluate the possible synergistic relationship between S-15 virus and Ascaris suum; (3) to detect the virus in swine lungworms. Progressive pathogenesis of S-15 virus in mice intranasally inoculated was followed by direct FA. The conjugate absorbed with swine liver powder and refractionated from DEAE cellulose column, was highly specific and sensitive for S-15 virus. In mice the infected cells were first detected 8 hours postinoculation, cytoplasmic and occasionally nuclear. FA reached a peak on the 2nd and 3rd day, spread to alveoli on the 2nd day, diminished after the 4th day, and disappeared by the 7th. Swine influenza virus progressively infects bronchi, bronchioles, alveolar ducts, and alveoli. Concentration of virus in the inoculum affected the distribution progression, and development of S-15 virus in mice inoculated intranasally. The time for appearance in mouse lungs decreased with increased concentration of virus. Egg infectivity titer and FA of virus in lungs correlated well. In specific pathogen-free pigs inoculated intranasally with S-15 virus, virus was detectable 2 to 4 hours postinoculation by FA. Clinical symptoms and pathology are described. Coinfection with migrating Ascaris larvae and S-15 virus had a synergistic effect in inducing enhanced mortality in mice, optimally when given 8 d . following Ascaris inoculation. Ascaris

migration appeared to break the blood-lung barrier for influenza virus. Infectious virus could not be detected in the lungworm extract in pigs or mice stressed with A. suum larvae.

6001

Sulzer, A.J. 1965. Indirect fluorescent antibody tests for parasitic diseases: I. Preparation of a stable antigen from larvae of Trichinella spiralis. J. Parasitol. 51:717-721.

A stable antigen was developed for the indirect fluorescence test for Trichinella spiralis antibodies. The antigen was prepared by digesting fresh larvae of T. spiralis with pepsin for 36 hours and fixing the cuticle in 10 per cent formol - 0.5 per cent bovine serum albumin. The antigen can be stored for several months without loss of activity or specificity. Evidence is advanced to support the hypothesis that specific antigenic sites are a constituent part of the larval cuticle.

6002

Sulzer, A.J.; Kagan, I.G. 1964. Fluorescent antibody (FA) test for schistosomiasis and trichinosis. J. Parasitol. 50(Suppl.):23-24.

The indirect FA test for schistosomiasis has been under investigation in this laboratory for several years and more recently the test for trichinosis. Techniques for rapid efficient methods of conjugating antiserum with FITC and the preparation of an antigen for trichinosis, stable under storage at 4 C, have been developed. Certain aspects basic to the FA reaction have been elucidated. One of the variables investigated was the effect of multiple washes of antigen after addition of positive serum and before addition of labeled antiglobulin conjugate. With some positive sera the FA test increased from a negative reaction with one wash to a brilliant 4 plus reaction with four washes. These results indicate that under certain conditions schistosome or trichina antigens that have been sensitized with gamma globulin from a positive serum will react weakly or negatively in the test. This effect may be overcome by use of antigen washed several times or by conjugate at relatively high concentration. This may clarify why a prozone effect has been reported in the FA test for trichinosis. Dependable methods were developed for production of labeled antiserum in less than 2 weeks from initial inoculation of the animal. Complete article.

6003

Taffs, L.F.; Voller, A. 1963. In vitro fluorescent antibody studies on Ascaris lumbricoides and Ascaris suum. Trans. Roy. Soc. Trop. Med. Hyg. 57:353-358.

The immunology of Ascaris suum and Ascaris lumbricoides was studied in vitro by the fluorescent antibody technique. The direct, indirect,

and combined methods of staining were used. A cross-reaction between the two ascarids was demonstrated by the fluorescence of decoated eggs of A. lumbricoides. Fluorescence of intact eggs was considered nonspecific. Circumoral precipitates did not form in either the homologous or heterologous immune systems. Cross-reactions with third-stage larvae were also observed when pig Ascaris larvae were placed in serum from monkeys immune to A. lumbricoides, and after larvae of this species were exposed to antisera from rabbits immune to the pig Ascaris. Fluorescent antibody was incorporated into precipitates at the mouse annus and on the cuticle of living larvae. In most instances the cuticle also fluoresced green. The digestive tract did not stain. The specificity of staining was confirmed. The results indicate that embryonated Ascaris eggs are antigenic. In third-stage Ascaris larvae, the antigenic sites are located at the natural orifices and cuticle. Worm eggs and third-stage larvae of A. lumbricoides and A. suum share common antigens. It has not been possible, so far, to differentiate the two worms by the fluorescent antibody technique.

6004

Taffs, L.F.; Voller, A. 1964. Fluorescent antibody staining of Ascaris suum larvae in the liver and lung of an infected guinea pig by means of the freeze-substitution technique. Ann. Trop. Med. Parasitol. 58:414-419.

By means of a freeze-substitution wax-embedding method, sectioned third-stage larvae of Ascaris suum, in the liver and lung of a guinea pig killed 7 days after experimental infection, were specifically stained with fluorescent antiserum by the direct and indirect fluorescent antibody techniques. Specific green fluorescence of the cuticle, digestive tract, and other internal tissues was observed. Common antigens between the third-stage larvae of both the hominid (A. lumbricoides) and the pig (A. suum) forms of Ascaris were demonstrated by the fluorescent staining of sectioned A. suum larvae after exposure to monkey anti-hominid Ascaris immune serum and to labeled goat antihuman globulin.

6005

Taffs, L.F.; Voller, A. 1965. Fluorescent antibody staining of Ascaris suum larvae in the liver and lung of an infected guinea pig by means of the freeze-substitution technique. Ann. Trop. Med. Parasitol. 58:414-419.

Direct and indirect FA was used to stain sections of 3rd stage larvae of A. suum. The sections were prepared by a wax-freeze-substitution method. The worms were in guinea pig lung and liver. Specific fluorescence of the cuticle, digestive tract, and other internal tissues was observed. Common antigens between the 3rd stage larvae of both the hominid (A. lumbricoides) and the pig (A. suum) forms of Ascaris were demonstrated by the fluorescent staining of sectioned A. suum larvae after exposure to monkey anti-hominid Ascaris immune serum and to labeled goat anti-human globulin.

6006

Zaman, V.; Chellappah, W.T. 1965. The application of fluorescent-antibody test to microfilariae of Brugia pahangi. Singapore Med. J. 6:40.

Brugia pahangi from cats was identified by FA, using FITC-conjugated rabbit antiserum. The antigenic relationship of different species of microfilariae is important especially when species are morphologically indistinguishable, as is the case for B. pahangi and B. malayi.

6007

Zaman, V.; Singh, M. 1965. Immuno-fluorescent studies with hookworms: I. Antigenic relationships of ova. Trans. Roy. Soc. Trop. Med. Hyg. 59:690-693.

FA was applied to ova of A. caninum and N. americanus, with sera from immunized rabbits. Fluorescence was clearly observed both around the embryonated and unembryonated ova, but their contents did not fluoresce. There was cross-reaction between A. caninum and N. americanus. The antisera against these two species also reacted against A. braziliense. The ova of Ascaris lumbricoides, Trichuris trichiura, and Dipylidium caninum showed no reaction either to A. caninum or N. americanus antisera. A. suum ova obtained from the uterus of gravid females showed nonspecific fluorescence.

C. SCHISTOSOMA

6008

Andrade, Z.A. 1964. Immunopathology of Manson's schistosomiasis. Rev. Inst. Med. Trop. Sao Paulo 6:181-187.

Reactions of the immediate type, involving circulating antibodies, are important in granulomatous formation around the S. mansoni ova in the tissues. They appear while the miracidium is still alive and exuding antigenic material. They become stronger after its death and disintegration. Immature eggs and heated mature eggs (dead miracidia) provoke little or not tissue reation. Circulating antibodies are active also against the other evolutive stages of the parasite, but since no antigenic material is actively eliminated, antigen-antibody complexes can only be formed after disintegration of adult worms. General antigenic stimulation can be observed in the splenic structure of infected mice. There is proliferation of reticular cells, plasma cell differentiation, and gamma globulin synthesis. Reactions of the delayed type are probably represented by the chronic portal hepatitis. This is described. Resistance can develop not only by limiting the parasitic factors but also by forming minimal and more effective inflammatory reactions to the parasitic stimuli. Such a situation can be observed in animals with prolonged infections.

6009

Andrade, Z.A.; Andrade, S.G. 1965. Pathology of the spleen in hepatosplenomegalic schistosomiasis. Rev. Inst. Med. Trop. Sao Paulo 7:218-227. In Portuguese.

A morphological study was made of spleens removed surgically from patients with hepatosplenic schistosomiasis mansoni. Histological, histochemical, and immunocytochemical studies were made. There are many more reticuloendothelial cells in the red pulp and in the white pulp than are noted using classical methods. Fusiform cells resembling fibroblasts in conventional sections had the histochemical reactivity of reticuloendothelial cells. Proliferation of fibroblasts was absent, and fibrosis of the splenic cords was seldom seen. Foci of plasma cells containing intracytoplasmic glycoprotein and synthesizing gamma globulin were frequently encountered. These findings reflect the profound involvement of the reticuloendothelial elements of the spleen in schistosomal splenomegaly and indicate the importance of the cellular factor in its pathogenesis. Congestion was present in all cases.

6010

Buck, A.A.; Sadun, E.H.; Anderson, R.I.; Shaffa, E. 1964. Comparative studies of some immunologic screening tests for schistosomiasis in Ethiopia. Amer. J. Hyg. 80:75-84.

A survey for schistosomiasis was conducted in 100 inhabitants of an Ethiopian village located in an area highly endemic for schistosomiasis (Schistosoma mansoni). Schistosomiasis was measured by the following: (a) a CF test; (b) a slide flocculation test; (c) a rapid card test; (d) an FA test; (e) a serologic test using the secretions and excretions of cercariae as antigens; (f) a skin test; and (g) stool examinations by several methods. The results of the immunodiagnostic tests were compared with those of the stool examinations. In contrast to the other tests where the results of the immunologic procedures and stool examinations were closely associated, the reactions of the FA test were independent of the presence or absence of S. mansoni eggs in the stool specimens of the individuals tested. This indicated a lack of specificity of this test. Although the indices measuring agreement were different for individual tests, two general trends can be recognized for all immunodiagnostic methods. Co-positivity increases with age whereas co-negativity decreases. The quality of the antihuman globulin conjugate used in the FA test might have been partially responsible for the relatively low specificity obtained.

6011

Cookson, L.O.C. 1963. Some investigations with the fluorescent antibody technique: I. Schistosomiasis. Cent. Afr. J. Med. 9:429-434.

FA diagnostic tests for schistosomiasis are described. Ova were not satisfactory antigens. Most consistent results were shown with cercaria and

miracidia. Cercaria were the best substrate for test. This test could be described as the minimal fluorescence or threshold fluorescence test.

6012

Cookson, L.O.C. 1963. Some investigations with the fluorescent antibody technique: II. Experiments with advanced fluorescent equipment. Cent. Afr. J. Med. 9:469-478.

These experiments confirmed previous findings in schistosome diagnosis. The microscopy equipment used is described in detail. Both the direct and the indirect FA tests proved to be sound tools for diagnosis of schistosomiasis. FA antiglobulin testing is probably the most sensitive and specific schistosomiasis test available. Disadvantages include inability to distinguish between species of schistosomes. Cross-antigenicity with other flukes such as Fasciola gigantica and F. hepatica was also demonstrated. Cercariae were the antigen of choice for the test.

6013

Cookson, L.O.C. 1964. Some modifications of the fluorescent antibody test in human bilharziasis. Bull. WHO 31:799-813.

Although the FA test for human bilharziasis has proved of great value, its use involves certain difficulties that the author has attempted to obviate. The paper describes a cheap and reproducible method for producing a cercarial antigen conjugated with rhodamine B 200 for use in the indirect FA test. The second part deals with a new modification in which the conjugated cercarial antigen is employed with a bentonite-absorbed FITC antihuman globulin serum and discusses the advantages of this test over the normal FA test. Experience has shown that the use of rhodamine-albumin-coated cercariae, conjugated cercariae, or normal fixed cercariae as antigens does not always give valid results when compared with those obtained with the FA test or the ordinary complement fixation test in bilharziasis. The author describes a modification of the complement fixation test involving the use of a bentonite-absorbed fluorescent anti-guinea-pig serum and the RB 200 - conjugated cercariae described earlier. This test has given reproducible results in known positive control human sera that have been valid when compared with the Sadun FA test, the conjugated cercarial FA test, and the bentonite fluorescent antibody test described in the second part of this paper.

6014

Cookson, L.O.C.; Clarke, V.; Pirie, E. 1964. Some investigations with the fluorescent antibody technique: III. The threshold fluorescent antibody test in schistosomiasis. Cent. Afr. J. Med. 10:12-16.

The principles of the fluorescent antibody technique are described, with the early investigations of this method in human schistosomiasis. Proof of the validity of the technique in schistosomiasis with advanced fluorescent microscopic equipment is described, with observations on the cross-antigenicity of related and unrelated parasites. The use of the method as an ideal diagnostic technique for schistosomiasis is discussed. Inexpensive and readily available microscopic equipment is described and discussed in the context of making the technique available for most routine laboratories for diagnosis of mammalian, including human, schistosomiasis.

6015

Foster, R. 1965. Skin antigen tests and the fluorescent antibody technique in the diagnosis of schistosomiasis. E. Afr. Med. J. 42:117-121.

Parallel tests of skin antigen and the fluorescent antibody technique for the diagnosis of schistosomiasis were carried out in Tanganyika. Both tests proved very sensitive in certain cases, but neither was infallible. The incidence of failure to detect infection by the skin test was higher than expected and the fluorescent antibody technique is considered to be a more reliable test. This may be due, at least in part, to a greater sensitivity of the fluorescent antibody technique to recently acquired infections.

6016

Filho, A.M.; Krupp, I.M.; Malek, E.A. 1965. Localization of antigen and presence of antibody in tissues of mice infected with Schistosoma mansoni, as indicated by fluorescent antibody techniques. Amer. J. Trop. Med. Hyg.14:84-99.

Fluorescent antibody techniques were used to determine the localization and distribution of Schistosoma mansoni antigen in tissue cells, the presence of circulating antibody, and the sites of antibody production or in vivo antigen-antibody combination. The experiments were performed in mice after a primary infection with cercariae, after several challenges, and after antigen stimulation and challenge. Evidence of the presence of circulating antibody was first observed at 20 days in the inhibition fluorescent antibody test, at 25 days in the indirect fluorescent antibody test, at 42 days with the cercarial fluorescent antibody test, and at 47 days in the Cercarienhullen reaction. Sites of antibody production or in vivo antigen-antibody combination were observed and are listed. The sites of antigen deposition after a single exposure were also recorded.

6017

Gane, N.F.C.; Hunt, A.L.C.; Booth, R.L. 1964. Some aspects of the fluorescent antibody test for bilharziasis. Cent. Afr. J. Med. 10:407-410.

Findings indicate that the FA test for bilharziasis, according to Sadun, is very satisfactory. A positive result is no measure of the activity of the disease.

6018

Jachowski, L.A., Jr.; Anderson, R.; Sadun, E.H. 1963. Serologic reactions to Schistosoma mansoni: I. Quantitative studies on experimentally infected monkeys, Macaca mulatta. Amer. J. Hyg. 77:137-145.

The complement-fixation, circumoval precipitin, slide flocculation, and fluorescent antibody tests applied to sera from monkeys experimentally infected with Schistosoma mansoni apparently did not indicate the immunologic status of the host. Sera of these monkeys remain reactive in these tests despite the disappearance of eggs from the feces. A positive correlation appears to exist between the antibody titer, especially in the complement fixation test, and the numbers of cercariae to which monkeys were exposed.

6019

Kagan, I.G.; Sulzer, A.J.; Carver, K. 1965. An evaluation of the fluorescent antibody test for the diagnosis of schistosomiasis. Amer. J. Epidemiol. 81:63-70.

Five batteries of sera were evaluated for schistosomiasis by the indirect fluorescent antibody test. Titration of sera from Puerto Rican males who had been carefully studied serologically, clinically, and parasitologically for infection with Schistosoma mansoni showed a sensitivity of 81 per cent for sera from infected individuals and low specificity, 40 per cent of the negative group were positive. Good reproducibility and sensitivity was obtained with 223 sera from individuals with schistosome and non-schistosome infections. The FA test was more sensitive than either the hemagglutination procedure for the detection of schistosome antibodies or the cholesterol-lecithin test. Titration of sera from individuals residing in nonendemic areas indicated a low degree of specificity for the FA test with some of the groups. Sera from two South Pacific islands were 63 per cent positive, from Alaska 22 per cent positive, from Manaus, Brazil, 25 per cent positive, and from the continental United States 14 per cent positive. These positive results were not influenced by trichinosis antibody, infection with filariasis, or intestinal parasitism. Further evaluation of this FA test is required.

6020

Pellegrino, J.; Biocca, E. 1963. Serological diagnosis of schistosomiasis mansoni: II. The immunofluorescence reaction with cercaria of Schistosoma bovis and Cercaria caratinguensis. Rev. Inst. Med. Trop. Sao Paulo 5:257-260. In Portuguese.

The immunofluorescence test for diagnosis of schistosomiasis was used with sera from patients with active schistosomiasis mansoni and with sera from noninfected individuals. Cercariae of S. bovis and Cercaria caratinguensis were used and the results were compared with those obtained with S. mansoni cercariae. It was concluded that these cercariae share common antigens.

6021

Peterson, W.P.; von Lichtenberg, F. 1965. Studies on granuloma formation: IV. In vivo antigenicity of schistosome egg antigen in lung tissue. J. Immunol. 95:959-965.

Recipient mice were sensitized intraperitoneally and subcutaneously with cell-free lung-granuloma homogenates obtained from donor mice at variable intervals following a single intravenous injection of purified eggs of Schistcsoma mansoni. The recipients were then challenged intravenously with intact schistosome eggs, and the percentages of pseudotubercles reacting with immunofluorescent antibody on the 8th day after challenge were compared between sensitized mice and their controls and among recipients of homogenates of lung granulomas ranging in duration from 1 to 180 days. These results were also compared with earlier data on immunofluorescent antibody titers. Using the immunofluorescent-stainable PAS-positive antigen produced by miracidia in the pseudotubercles as an indicator, it was shown that lung granuloma homogenates remained sensitizing through the 30th day of the evolution of the primary pseudotubercle. Antigen sequestration results in a gradient of antigen concentration between the granuloma center and the milieu interne of the host.

6022

Radke, M.G.; Sadun, E.H. 1963. Resistance produced in mice by exposure to irradiated Schistosoma mansoni cercariae. Exp. Parasitol. 13:134-142.

An acquired resistance to Schistosoma mansoni was observed in mice following a previous exposure to irradiated cercariae. The resistance developed was sufficiently strong to eliminate or to prevent the development of a significant number of worms of the test dose, no matter whether the primary infection was as low as 200 or as high as 15,000 cercariae, or whether the immunizing infection was in a single dose or in three weekly doses. Resistance induced by a single exposure to irradiated cercariae was sufficiently powerful to protect mice from an otherwise lethal dose of normal cercariae. Mice stimulated with irradiated cercariae developed detectable antibodies to egg and larval antigens in spite of the fact that they

received no egg stimulation. Indirect FA was one of the methods used for antibody titration.

6023

Rivera de Sala, A.; Cancio, M.; Rodriguez-Molina, R. 1965. Ion exchange chromatography in the separation of circumoval precipitins. Bol. Asoc. Med. Puerto Rico 57:210-212.

A simplified procedure has been developed for the isolation of the protein fraction containing the circumoval precipitin antibodies from the serum of patients infected with Schistosoma mansoni. The procedure is eight to ten times faster than other procedures previously used and makes unnecessary the use of special laboratory facilities. The globulins obtained have been utilized for the performance of the fluorescent circumoval precipitin technique.

6024

Sadun, E.H.; Anderson, R.; DeWitt, W.; Jachowski, L.A., Jr. 1963. Serologic reactions to Schistosoma mansoni: II. Quantitative studies in human patients treated with stibophen. Amer. J. Hyg. 77:146-149.

Patients receiving stibophen ceased passing eggs following therapy, but serum antibodies remained up to 10 months following therapy. No living eggs were detected by stool examinations repeated for a period of 5 months after treatment. Since the adult worms could have been sterilized and still survived, the failure to detect eggs in fecal or tissue specimens is not proof of cure. High titers to cercarial antigen were obtained by the FA test.

6025

Sadun, E.H.; Bruce, J.I. 1964. Resistance induced in rats by previous exposure to and by vaccination with fresh homogenates of Schistosoma mansoni. Exp. Parasitol. 15:32-43.

Rats developed an acquired resistance to Schistosoma mansoni following previous injections with whole-worm homogenates. This resistance was manifested by a significant reduction in the number of developing worms from the challenging exposure and was observed whether the rats received three or two inoculations of worm homogenates. Resistance to superinfection was also observed when 1- and 14-day-old rats were exposed to a primary infection. In three of four experiments rats inoculated with albumin developed a significantly increased resistance to S. mansoni that was comparable in some instances to that developed by the animals that received worm homogenates. Serologic studies indicated that the rats that received worm homogenates developed detectable levels of fluorescent antibodies earlier than their normal controls.

Similarly, some rats that received albumin developed detectable antibodies earlier than the controls. Indirect FA was employed for antibody detection.

6026

Sato, S.; Imamura, S.; Yoneyama, K. 1964. Fluorescent antibody studies of Schistosoma japonicum infections. Gunma J. Med. Sci. 13:199-205.

An investigation was conducted to study the mechanisms of the phenomenon of the FA test for schistosomiasis. When living cercariae were treated by direct FA, fluorescence was observed on the precipitates around the body, not on the precipitates from the oral sucker of the cercaria. Living cercariae treated by indirect FA showed fluorescence both on the membraneous envelope produced by the circum-cercarial reaction and on the precipitates formed around the anterior organ. Different mechanisms of reactions were responsible for results by the direct and indirect tests.

6027

Sulzer, A.J.; Kagan, I.G. 1964. Fluorescent antibody (FA) test for schistosomiasis and trichinosis. J. Parasitol. 50(Suppl.):23-24.

The indirect FA test for schistosomiasis has been under investigation in this laboratory for several years and more recently the test for trichinosis. Techniques for rapid efficient methods of conjugating antiserum with FITC and the preparation of an antigen for trichinosis, stable under storage at 4 C, have been developed. Certain aspects basic to the FA reaction have been elucidated. One of the variables investigated was the effect of multiple washes of antigen after addition of positive serum and before addition of labeled antiglobulin conjugate. With some positive sera the FA test increased from a negative reaction with one wash to a brilliant 4 plus reaction with four washes. These results indicate that under certain conditions schistosome or trichina antigens that have been sensitized with gamma globulin from a positive serum will react weakly or negatively in the test. This effect may be overcome by use of antigen washed several times or by conjugate at relatively high concentration. This may clarify why a prozone effect has been reported in the FA test for trichinosis. Dependable methods were developed for production of labeled antiserum in less than 2 weeks from initial inoculation of the animal. Complete article.

6028

von Lichtenberg, F. 1964. Studies on granuloma formation: III. Antigen sequestration and destruction in the schistosome pseudotubercle. Amer. J. Pathol. 45:75-94.

Eggs of Schistosoma mansoni were studied by the indirect FA to ascertain distribution and relative amount of stainable schistosome antigen in vitro at various stages of host reaction. Worms and eggs in mammalian tissue

showed selective immunofluorescent staining. A major egg antigen produced by miracidia contained polysaccharide and could represent the vehicle of cercarial snail-penetrating hyaluronidase. Deposits were labeled in penetration glands, along the eggshell, and in small granules in phagocytes of the pseudotubercle. The antibody responsible for immunofluorescence was related to circumoval precipitate. After intravenous infection of eggs into nonsensitized mice, four successive stages of antigen disposal could be distinguished. These stages are outlined in detail. In mice presensitized by intraperitoneal egg injection, both antigen disposal and host cell reaction were accelerated. Antigen sequestration appeared to be enhanced and the antigen disappeared prior to the 32nd day. In the naturally infected mouse liver, antigen destruction was completed within 34 days of oviposition. Hypotheses and findings were the result of a balance between antigen release by eggs and phagocytic-catabolic activity in host cells. Secondary pseudotubercle formation was an enhanced response. The pathogenetic significance of these concepts is discussed.

6029

von Lichtenberg, F.; Sadun, E.H.; Bruce, J.I. 1963. Host response to eggs of Schistosoma mansoni: III. The role of eggs in resistance. J. Infect. Dis. 113:113-122.

A single intravenous injection of purified eggs of Schistosoma mansoni had no effect on the number, size, sex ratio, and egg production of adult worms arising from a standard challenge with 200 cercariae. Nevertheless, tissue changes in the lung and spleen indicative of sensitization were still detectable 50 days following the challenge, and these included enhancement of pulmonary arteritis and of splenic hyperplasia and eosinophilia. Cercarial antibodies appeared in the serum after egg injection and before cercarial challenge. On critical analysis of these findings and of the literature it is concluded that the role of the egg in resistance to Schistosoma is negligible and that the role of the schistosomulum is paramount. The findings present an interesting example of dissociation between the phenomena of sensitization and resistance. Circulating antibody was demonstrated by the cercarial FA test.

6030

Yokogawa, M. 1964. Immunological study of parasites. Naika Hokan 13:92-98. In Japanese.

The criteria for intradermal and complement fixation tests for lung fluke disease, schistosomiasis, and other parasitic diseases are discussed. Practical value of the precipitin test is small. In immunobiological diagnoses, parasitic larvae of a certain age are used as antigens and diagnosis is made by observing the changes in the larvae. Among the tests studied are the Sarles phenomenon, miracidial immobilization, circumoval precipitin test, cercarial Huellen reaction, cercarial agglutination test, and the FA test. FA is convenient for collective testing.

III. PROTOZOA

A. AMOEBAE

6031

Albach, R.A.; Shaffer, J.G.; Watson, R.H. 1965. Morphology, antigenicity, and nucleic acid content of the Bacteroides sp. used in the culture of Entamoeba histolytica. J. Bacteriol. 90:1045-1053.

Certain changes are described in morphology, antigenicity, and nucleic acid content that occur in a culture of Bacteroides sp. in the presence of penicillin G in CLG medium. This variant is one of seven recovered in several laboratories, all of which are descendants of the original Bacteroides isolated by Shaffer and Frye. Penicillin-inhibited cells of this culture are currently being used in the routine propagation of Entamoeba histolytica in CLG medium. Evidence is presented for the loss of ability to react with antibody in these penicillin-inhibited bacteria in CLG medium, when studied by fluorescent antibody techniques. The implications of the antigenic changes observed as they pertain to similar antigenic studies of the amoebas are discussed. A pronounced reduction in the ribonucleic acid (RNA) content of such penicillin-inhibited cells was also observed. The potential importance of the changes that occur in the RNA of these cells with respect to considerations of the growth requirements of the amoebas is also discussed.

6032

Jeanes, A.L. 1964. Immunofluorescent diagnosis of amoebiasis. Brit. Med. J. 2:1531.

Indirect FA was used to detect antibodies in Entamoeba histolytica in human sera.

6033

Jeon, K.W.; Bell, L.G.E. 1964. Behavior of cell membrane in relation to locomotion in Amoeba proteus. Exp. Cell Res. 33:531-539.

The behavior of the mucous coat of A. proteus was studied by the fluorescent-labeling technique. It has been shown that the mucous coat is fluid and flows forward over the newly formed pseudopodia. Further, this coat can be stripped off the amoeba or prevented from flowing forward. These findings are interpreted to indicate the separate behavior of the mucous coat and the cell membrane during normal locomotion in amoebae. It is concluded that there is, as yet, no experimental evidence to show the behavior of the cell membrane itself.

6034

Magaudda-Borzi, L. 1962. Antigenic structure of Entamoeba histolytica and correlation of serological investigations. G. Mal Infett. 14:174-215. In Italian.

As a part of this study, several amoebae were differentiated by FA.

6035

Zaman, V. 1965. The application of fluorescent antibody test to cysts of Entamoeba invadens. Experientia 21:357-358.

The tests were performed using cysts prepared by various methods. The test slides showed intense fluorescence when both the unfixed and chemically fixed cells were used. None of the fixatives destroyed the specificity of the reaction. Nonspecific staining was negligible in unfixed cells and only slight in the chemically fixed cells. Formalin fixation seemed to be the best. It least affected the specificity of the reaction. However, acetone gave the brightest fluorescence.

B. ANAPLASMA

6036

Amerault, T.E.: Roby, T.O. 1964. An exo-antigen of Anaplasma marginale in serum and erythrocytes of cattle with acute anaplasmosis. Amer. J. Vet. Res. 25:1642-1647.

Precipitating antibodies of Anaplasma marginale were detected by an agar double diffusion (AD) technique, using complement-fixation (CF) antigen and sera from acutely infected and carrier cattle. These antibodies appeared late in the acute phase of anaplasmosis, as contrasted to CF antibodies, and persisted or recurred for irregular interva during the carrier phase. By the AD technique, a soluble Anaplasma exo-antigen was found free in sera and in red blood cells (RBC) of cattle with acute anaplasmosis. The presence of this antigen was related to the peak of Anaplasma-parasitized RBC and was usually detected 1 or 2 days before maximum parasitemia.

6037

Anthony, D.W.; Madden, P.; Gates, D. 1964. Anaplasma marginale Theiler observed in the gut and excreta of Dermacentor andersoni Stiles or Dermacentor venustus Marx. Amer. J. Vet. Res. 25:1464-1472.

Dermacentor andersoni specimens were allowed to feed on splenectomized calves infected with Anaplasma marginale and on a splenectomized calf free of anaplasmosis. Tissues and excreta from the ticks were studies by immuno-fluorescence, bright-field, and electron microscopy. Anaplasmata were found by immunofluorescent methods in smear preparations of gut and excreta preparations were found by bright-field and electron microscopic examinations. Upon electron microscopic examination of ultra-thin sections

through gut diverticula, structures believed to be A. marginale were seen in undigested erythrocytes. Anaplasmata were not found, however, in ultra-thin sections of the salivary glands and reproductive organs.

6038

Kreier, J.P.; Ristic, M. 1963. Anaplasmosis: VII. Experimental Anaplasma ovis infection in white-tailed deer (Dama virginiana). Amer. J. Vet. Res. 24:567-572.

Two Virginia white-tailed deer (Dama virginiana) were inoculated with blood from a sheep infected with Anaplasma ovis. By examination of blood smears stained with Giemsa's stain and fluorescein-labeled antibody and by subsequent inoculation of sheep and a calf, it was found that the deer had become infected with A. ovis.

6039

Kreier, J.P.; Ristic, M. 1963. Anaplasmosis: X. Morphologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of Anaplasma marginale. Amer. J. Vet. Res. 24:676-687.

An Oregon strain of Anaplasma marginale was studied in cattle erythrocytes and compared with a Florida strain. Erythrocytes of cattle infected with the Florida strain contained only one morphologic class of parasites. Those from cattle infected with Oregon strain contained two major morphologic classes and a third morphologic class that was seen rarely and irregularly. These three morphologic classes of parasites could only be identified when the organisms were studied after preparation by special stains and techniques, including FA. The predominant class of organisms in the Oregon strain of Anaplasma was morphologically identical to the parasites of the Florida strain. These parasites appeared as round marginal bodies by all methods of study employed. The other two classes of parasites either had a marginally situated head, a body, and a long tail or were shaped like a bipolar disc. The bodies and tails of the tailed parasites and the bodies of those shaped like bipolar discs had no affinity for acridine orange or Giemsa stains, whereas the heads stained.

6040

Kreier, J.P.; Ristic, M. 1963. Anaplasmosis: XI. Immunoserologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of Anaplasma marginale. Amer. J. Vet. Res. 24:688-696.

The antigenic and immunoserologic characteristics of the two principal classes of parasites that occur in the erythrocytes of cattle infected with the Oregon strain of Anaplasma were studied and compared with those of the parasites that occur in the erythrocytes of cattle infected

with the Florida strain. It was demonstrated by means of FA studies and cross-immunity studies with preimmune cattle that the round marginal body class of parasites in the erythrocytes of cattle infected with the Oregon strain of Anaplasma is antigenically identical to the parasites in the erythrocytes of cattle infected with the Florida strain. The class of parasites characterized by a head, a body, and a long tail in the erythrocytes of cattle infected with the Oregon strain was antigenically and serologically distinct.

6041

Ristic, M.; Mann, D.K. 1963. Anaplasmosis: IX. Immunoserologic properties of soluble Anaplasma antigens. Amer. J. Vet. Res. 24:478-482.

A precipitating antibody occurring in cattle with anaplasmosis was revealed by subjecting soluble Anaplasma antigens to reaction with sera of these cattle in a gel diffusion test. In an immunoelectrophoretic system, the soluble antigen migrated toward the positive pole. On the basis of studies by cross-absorption, precipitation, and agglutination tests, there was no antigenic relationship between the corpuscular and the soluble Anaplasma antigens. The sequence of tests in which individual types of antibodies appeared in serum of a calf with acute infection was as follows: complement fixation, followed by capillary tube agglutination, and finally by gel precipitation. Sheep erythrocytes sensitized with the soluble Anaplasma antigens were used in a passive hemagglutination test for detection of an antibody present in serum of infected cattle. There was no observable skin reaction in two Anaplasma-carrier calves given intradermal injections of soluble antigens.

6042

Ristic, M.; Watrach, A. 1963. Anaplasmosis: VI. Studies and a hypothesis concerning the cycle of development of the causative agent. Amer. J. Vet. Res. 24:267-277.

Daily observations of bovine blood were made by FA and electron microscopic techniques during the course of an experimental Anaplasma infection. Based upon the frequency of occurrence of Anaplasma and its localization in the infected erythrocytes, four developmental stages of the organism were observed through use of FA: early stage, consisting of initial bodies; mixed population stage, consisting of marginal and initial bodies; vigorous growth and transfer stage; and massive multiplication stage with a predominance of marginal bodies. Normal and infected erythrocytes were observed by electron microscopy. The first initial Anaplasma bodies were observed on the 5th day following infection. The first marginal Anaplasma bodies, composed of two to three initial bodies, were observed on the 10th day postinfection. Later, marginal bodies consisted of eight to ten initial bodies. The invasive mechanism of Anaplasma as revealed by electron microscopy involved penetration of the erythrocytic membrane by initial bodies. The initial Anaplasma body reproduces by binary fission. Four

morphologically distinct stages in the division sequence were observed. A complete developmental cycle of the organism occurs in the mature erythrocytes. Binary fission of the initial body and its direct transfer between erythrocytes fully explains the mode of development of this agent.

6043

Rogers, T.E.; Dimopoullos, G.T. 1963. Studies on the antigens of Anaplasma marginale. Bacteriol. Proc. V48:140.

Anaplasma-infected erythrocytes were studied by chemical fractionation and differential and density gradient sedimentation. Various fractions were examined for CF activity, for reaction with anti-Anaplasma fluorescent antibody, and by electron microscopy. By chemical fractionation, antigenic activity was found to be associated with the elinin fraction of the infected erythrocyte. Activity in high titer was found in a fraction prepared by differential centrifugation of sonically disrupted erythrocytes. The density of the fraction containing most of the CF activity was high and the material did not react with fluorescent antibody. The fraction that reacted with fluorescent antibody did not possess CF activity. Purified preparations of marginal bodies did not exhibit significant CF antigenic activity or specific fluorescence, but fractions containing only erythrocytic cellular debris exhibited high CF antigenic activity and fluorescence. These immunofluorescence studies have revealed that a fairly loose association exists between two fractions of Anaplasma inclusion bodies: a reactive coating and a more dense nonreactive portion.

6044

Rogers, T.E.; Dimopoullos, G.T. 1964. Nature of the Anaplasma CF antigen. Bacteriol. Proc. M218:85.

Initial attempts to resolve the CF antigen from crude stromatal antigens of A. marginale by chemical and physical methods indicated that the CF antigen was a lipoprotein. A method developed for the preparation of a highly desirable Anaplasma CF antigen involved disintegration of infected erythrocytes by sonic vibration and separation of the antigen by differential centrifugation. Antigens prepared by this method were highly specific, colorless, did not exhibit anticomplementary activity, and possessed much higher titers than standard Anaplasma CF antigens. Qualitative biochemical tests and enzyme sensitivity studies indicated the antigen to be lipoprotein. Electron microscopic observations of the antigen produced from sonic extracts of infected erythrocytes showed that the preparation was not composed of Anaplasma bodies but of amorphous membranous material. The material reacted specifically when stained with anti-Anaplasma fluorescein-conjugated globulin. Data obtained by the CF test and the fluorescent antibody technique suggested that an antigenic matrix envelops the Anaplasma body and may be disrupted by physical means. This coat appears to be responsible for the CF antigenic activity.

6045

Schroeder, W.F.; Ristic, M. 1965. Anaplasmosis: XVIII. An analysis of autoantigens in infected and normal bovine erythrocytes. Amer. J. Vet. Res. 26:679-682.

Free-serum autoantibody was absorbed by a soluble antigen obtained from normal and Anaplasma-infected erythrocytes and by normal erythrocytes treated with trypsin. A reaction between free-serum autoantibody and trypsin-treated erythrocytes from Anaplasma-free cattle was shown by serologic absorption, hemagglutination, and indirect fluorescent antibody techniques.

6046

Summers, W.A. 1965. A rapid procedure for preparing fluorescein-isothiocyanate-labeled anti-anaplasma gamma globulin. Amer. J. Vet. Res. 26:1459-1462.

A method for the conjugation of anti-anaplasma bovine gamma globulin with FITC was described. This procedure reduced the chance of protein denaturation, decreased the time required for preparation, and resulted in a product that imparted brilliant fluorescence when applied to Anaplasma marginale in infected erythrocytes.

6047

Welter, C.J. 1964. Serologic stability and specificity of agglutinating Anaplasma marginale antigen. Amer. J. Vet. Res. 25:1058-1061.

The antigen used in the capillar tube-agglutination (CA) test for anaplasmosis was stable after prolonged exposure to pH 4.8 to 8.0, 0.2 to 0.5 per cent formalin, 0.2 to 1.0 per cent phenol, and 16.0 per cent NaCl. Thermostability of CA antigen was demonstrated at 45 C for 3 months, 37 C for 6 months, 4 C for 20 to 25 months, and 25 C for 24 months. No cross-reactions between Anaplasma CA antigen and antibodies specific for 21 other pathogens were observed. Cross-reactions were observed between CA antigen and antibodies to Anaplasma ovis in ten experimentally infected sheep. A globulin fraction was separated from sera of cattle naturally infected with Anaplasma and conjugated to fluorescein. The Anaplasma bodies of the CA antigen became fluorescent when exposed to conjugated immune globulin. The ability of unlabeled CA-positive sera and the inability of the CA-negative serum to block the fluorescent antibody reaction confirmed specificity and accuracy of the CA test.

C. LEISHMANIA

6048

Bray, R.S.; Lainson, R. 1965. The immunology and serology of leishmaniasis: I. The fluorescent antibody staining technique. Trans. Roy. Soc. Trop. Med. Hyg. 59:535-544.

Indirect FA was used to stain smears of the leptomonads of ten strains of Leishmania spp. including L. donovani, L. infantum, L. tropica, L. braziliensis braziliensis, L. braziliensis pifanoi, and L. mexicana, and the Leishman-Donovan bodies of some strains. The sera used successfully and giving a strong reaction were from rabbits artifically immunized by leptomonads and human sera from patients infected with Mediterranean infantile kala-azar, South American kala-azar, South and Central American muco-cutaneous, or cutaneous leishmaniasis. Other sera described were used successfully but gave weak reactions. These included sera from patients with leishmaniasis tegumentaria diffusa, uta and British Honduran chiclero's ulcer, and serum from monkeys infected with L. mexicana and L. braziliensis. Reactions were weaker with Leishman-Donovan bodies as slide antigen than with leptomonads. No differentiation of strains was possible. A group antigen-antibody reaction was shown by FA. Specific absorption of sera completely eliminated the reaction regardless of the antigen used for absorption. Sera from patients with chiclero's ulcer, non-immune subjects and others, were tested for antibodies by FA. No correlation was obtained between disease or possible exposure and positive FA results. The FA test is not useful for the detection of past or present infection with L. mexicana.

6049

Convit, J.; Kerdel-Vegas, F. 1965. Disseminated cutaneous leishmaniasis. Arch. Dermatol. 91:439-447.

We have studied a form of leishmaniasis (leishmaniasis cutis diffusa) that we consider to be a variety of American cutaneous leishmaniasis with clinical, immunological, parasitological, and ovolate characteristics of its own that distinguish it from the common American cutaneous type. We believe the causative agent to be a variant of L. braziliensis with characteristics specific for its strain. Leishmania treated with serum from patients of the disseminated cutaneous form of leishmaniasis did not fluoresce in the indirect FA test. Sera from patients of the common American form did fluoresce. Serologic differences between the forms of the disease were thus demonstrated.

6050

Duxbury, R.E.; Sadun, E.H. 1963. Fluorescent antibody test for serological diagnosis of visceral leishmaniasis in humans. J. Parasitol. 49(Suppl.):57.

Indirect FA was used and evaluated in the serodiagnosis of kala-azar in humans. The tests were performed on slides using Leishmania donovani leptomonad forms as antigen. Evans blue, employed as a counterstain, resulted in improved contrast, thus making the test easier to interpret, yet did not significantly diminish the specific yellow-green fluorescence. Of 30 specimens from proven kala-azar infections, all but four reacted with L. donovani. Conversely, of 22 serum specimens from healthy controls, only one gave a weak reaction in this test. Of 92 specimens from individuals with other parasitic and bacterial infections, 77 did not react in this test. Cross-reactions occurred with other Leishmania sera (8 of 19 specimens), and a few were obtained with sera of patients with Chagas' disease and malaria. The efficacy of the filter paper collection and extraction method developed in this laboratory for other parasitic infections was also evaluated. Complete article.

6051

Duxbury, R.E.; Sadun, E.H. 1964. Fluorescent antibody test for the serodiagnosis of visceral leishmaniasis. Amer. J. Trop. Med. Hyg. 13:525-529.

The indirect fluorescein-labeled antibody technique was used and evaluated in the serodiagnosis of kala-azar in humans. The tests were performed on slides using Leishmania donovani leptomonad forms as antigen. Evans blue, employed as a counter stain, resulted in improved contrast, thus making the test easier to interpret, yet did not significantly diminish the specific yellow-green fluorescence. Occasional cross-reactions were observed with specimens from individuals with viral, bacterial, mycotic, and parasitic infections as well as with degenerative diseases. Although cross-reactions were frequently observed in individuals with mucocutaneous leishmaniasis, negative results were reported from L. tropica patients. Reliable testing for visceral leishmaniasis fluorescent antibodies was possible even when dried blood smears were used instead of serum.

6052

Herman, R. 1964. Fluorescent antibody studies with the intracellular form of Leishmania donovani in cell culture. J. Parasitol. 50(Suppl.):18.

In vitro immunological study of Leishmania donovani poses certain problems connected with the parasite's intracellular position in the mammalian host. As a possible solution to these problems, Leishman-Donovan bodies (LDs) were grown in hamster macrophages in cell culture. To obtain infected macrophages, saline-stimulated hamsters were reinoculated intraperitoneally with a partially purified suspension of LDs from an infected hamster's spleen. After 30 hours, infected macrophages were harvested with a Hanks "SS 60 per cent inactivated serum 40 per cent medium and explanted into Leighton tubes, each containing a cover slip. After 1 or 2 days at 37 C, the cover slips were removed from the tubes and air dried. Both fixed (cold acetone) and nonfixed cover slips from such cultures were stored at -20 C. Cover slips with infected cells were well suited as antigens

for the demonstration of antibody in immune globulin by FA. Infected mice provided the immune globulin. Normal mouse globulin served for the controls. Using both direct and indirect FA, antibodies were successfully demonstrated, but not until after the 16th day. The cell culture - FA combination may help solve some diagnostic and other problems in leishmaniasis. Complete article.

6053

Herman, R. 1965. Fluorescent antibody studies on the intracellular form of Leishmania donovani grown in cell culture. Exp. Parasitol. 17:218-228.

Leishman-Donovan bodies of Leishmania donovani from an infected hamster's spleen were injected intraperitoneally into the previously saline-stimulated abdominal cavities of hamsters. Macrophages containing intracellular parasites were harvested from these hamsters and maintained in vitro on cover slips in Leighton tubes in a balanced salt solution - serum medium. These parasitized cells on cover slips were used as the antigen in FA studies. The parasitized cells were used to detect and titrate the anti-leishmanial antibody from sera of infected mice by both the direct and indirect FA techniques. These infected macrophages were also used to detect and titrate the anti-leishmanial antibody in sera from proven cases of kala-azar.

6054

Oddo, F.G.; Cascio, G. 1963. The immunofluorescent test in visceral and cutaneous leishmaniasis. Riv. Ist. Sieroterap. Ital. 38:139-145. In Italian.

The results of fluorescent antibody tests on serum specimens from four children with visceral leishmaniasis and three adults with cutaneous leishmaniasis are reported. Sera from a healthy child and adult, as well as sera from two hypergammaglobulinemic children were used as controls. The sera from the patients with leishmaniasis all showed clearly positive results. The controls were negative, with only a very slight fluorescence in the lower dilutions of the hypergammaglobulinemic sera.

6055

Shuikina, E.E. 1965. Use of the indirect fluorescent antibody method in a study of cutaneous leishmaniasis. Med. Parazitol. Parazitarn. Bolezni 34:5:576-582. In Russian.

By agglutination test and indirect FA investigations were carried out with 13 sera from persons who had suffered from vaccination cutaneous leishmaniasis of the rural type, six sera from guinea pigs infected with Leishmania enrietti, and sera from two rabbits, one of which was

immunized with culture of L. tropica major, strain P and the other with L. enrietti. For control purposes ten sera from people without history of cutaneous leishmaniasis and from six intact guinea pigs were taken. By FA in human and guinea pig sera, antibody was found in titers 1:10 to 1:50, in rabbit sera not lower than 1:1600. The agglutination test was poorly specific.

D. PLASMODIUM

6056

Abele, D.C.; Tobie, J.E.; Hill, G.J.; Contacos, P.G.; Hornick, R.B. 1964. Alterations in serum proteins and antibody production during human infections. Federation Proc. 23:1475:347.

Certain alterations in plasma protein immunoelectrophoretic patterns were observed in sera of volunteers infected with Plasmodium vivax. No significant changes were noted in volunteers infected with Salmonella typhi. Among the immunoelectrophoretic changes observed during the course of the malarial infections there was a consistent, marked increase in the beta-2 macroglobulins. This increase appeared temporally related to the first appearance of antimalarial antibodies as detected by the indirect fluorescent antibody technique and persisted in some cases as long as 60 days. A number of sera have been separated by Sephadex G-200 filtration and the fractions tested for antibody activity. The results indicate that the increase in beta-2 macroglobulin is partially due to the presence of antimalarial antibodies in this serum component. Data also suggest that early antimalarial antibody is predominantly a beta-2 macroglobulin and that later antibody is predominantly a 7S gamma globulin. Complete article.

6057

Abele, D.C.; Tobie, J.; Hill, G.; Contacos, P.; Evans, C. 1965. Alterations in serum proteins and 19S antibody production during the course of induced malarial infections in man. Amer. J. Trop. Med. Hyg. 14:191-197.

Increases in beta-2M macroglobulins have been detected by immunoelectrophoresis during the course of induced malarial infections in man. The increase in this serum globulin coincides closely with the appearance of antibodies to malaria as detected by fluorescent antibody studies. Evidence is presented that 19S and beta-2M macroglobulin antibodies as well as 7S gamma globulin antibodies are formed during the course of primary malarial infections in human volunteers.

6058

Ciuca, M.; Ciplea, A.G.; Bona, C.; Pozsgi, N. 1965. Degradation of malarial antigen by immunogenesis: Functions of humoral and cellular factors in acquired immunity. Pathol. Microbiol. 28:668-682. In French.

The factors of specific and nonspecific cellular and humoral defense are shown to be permanently linked to the mechanisms of degradation of malarial antigen. In a first phase, the phagocytic activity of the macrophages of the RES is predominant, but in a second phase, in individuals becoming immune the destruction of the parasites is assured by the synergistic intervention of antibodies synthesized by cells of the lymphocyte-plasmocyte type and by macrophages, which remain intensely active in their phagocytic and metabolic activity.

6059

Collins, W.E.; Jeffery, G.M.; Skinner, J.C. 1964. Fluorescent antibody studies in human malaria. Amer. J. Trop. Med. Hyg. 13:15.

The production and persistence of specific antibody was demonstrated by the indirect fluorescent antibody method in three patients inoculated with blood parasites of Plasmodium malariae. In one case a relatively high antibody level was maintained in association with persistent asymptomatic parasitemia; in one of the others, termination of the infection resulted in a delayed, slow decline in antibody titer to a persistent lower level. Higher antibody titer appeared to be associated with the primary infection characterized by reduced parasitemia and febrile attack.

6060

Collins, W.E.; Jeffery, G.M.; Skinner, J.C. 1964. Fluorescent antibody studies in human malaria: II. Development and persistence of antibodies to Plasmodium falciparum. Amer. J. Trop. Med. Hyg. 13:256-260.

The production and persistence of specific antibody was demonstrated by the indirect fluorescent antibody method in patients inoculated with blood parasites of Plasmodium falciparum. With P. falciparum, Thailand strain, antibody developed in response to the presence of patent parasitemias and the titers fluctuated significantly during the primary attack, apparently in a delayed direct association with fluctuation in parasite densities induced by noncurative dosages of antimalarial drugs. The rapid antibody response and long antibody persistence in the one patient with P. falciparum, Colombia strain, indicated either a possible previous exposure to malaria or a response peculiar to that strain of P. falciparum. Antibodies to the Colombia strain persisted for as long as 20 months after sporozoite inoculation with little or no decrease in antibody titer between 12 and 20 months.

6061

Collins, W.E.: Jeffery, G.; Skinner, J. 1964. Fluorescent antibody studies in human malaria: III. Development of antibodies to Plasmodium falciparum in semi-immune patients. Amer. J. Trop. Med. Hyg. 13:777-782.

Using the indirect fluorescent antibody (FA) method, the production of antibody to Plasmodium falciparum was demonstrated in eight patients having prior experience with strains of the parasite. The FA endpoints rose rapidly to levels much higher than had been reported for patients who had had no previous experience with P. falciparum. In two patients, FA response was such that it was possible to differentiate between endpoints to the Colombia and Thailand strains of P. falciparum.

6062

Collins, W.E.; Skinner, J.C.; Guinn, E.G.; Dobrovolny, C.G.; Jones, F.E. 1965. Fluorescent antibody reactions against six species of simian malaria in monkeys from India and Malaysia. J. Parasitol. 51:81-84.

Sera from 45 Malaysian and 51 Indian monkeys were tested for fluorescent antibody response to antigens of Plasmodium fieldi, P. gonderi, P. inui, P. coatneyi, P. knowlesi, and P. cynomolgi bastianellii. A total of 17 Malaysian and 33 Indian monkey sera had positive antibody responses, with the larger number reacting with P. fieldi and P. gonderi. The results suggest the possibility of prior malaria infection or of nonspecific antibody to parasites. The intensity of this response to heterologous or common antigens may be sufficient to mask a species-specific response. The use of monkeys having no initial fluorescent antibody titer for Plasmodium is suggested for studies involving antibody in monkey malaria.

6063

Corradetti, A.; Verolini, F.; Sebastiani, A.; Proietti, A.M.; Amati, L. 1964. Fluorescent antibody testing with sporozoites of plasmodia. Bull. WHO 30:747-750.

Staining sporozoites of plasmodia with fluorescent antibodies opens the way to research that may have practical importance. The fluorescent antibody staining appears to be specific and it may therefore prove possible to identify sporozoites found in mosquitoes captured in nature.

6064

Curtain, C.C.; Baumgarten, A. 1965. The distribution of genetic factors in malaria antibodies as determined by a fluorescent antibody test. Australian J. Exp. Biol. Med. Sci. 43:351-358.

The malaria antibody of five of 34 Melanesian Gm (a b) donors was found to possess no Gm (a) or Gm (b) specificity. In three others the malaria antibody possessed Gm (a) specificity only and in one Gm (b) specificity only. Eighteen of the 34 donors were Inv (a) and the malaria antibody possessed no Inv (a) specificity. It was suggested that phenotypic restriction of the malaria antibody might occur if the individual's initial experience of malaria in infancy occurred before all of the gamma globulin allotypes had developed. (Note: Letters in parentheses refer to genetic markers.)

6065

Curtain, C.C.; Gorman, J.G.; Kidson, C. 1965. Malaria antibody and gamma globulin levels in Melanesian children in New Guinea. Trans. Roy. Soc. Trop. Med. Hyg. 59:42-45.

A correlation was attempted between serum gamma globulin and malaria antibody levels in two groups of children living in coastal New Guinea, exposed to different intensities of malaria infection. Fewer children in the protected group possessed malaria antibody and the mean titers were lower than in the unprotected group. Only small differences could be detected between the gamma globulin levels. As the protected group was still subject to the other endemic infections prevalent in coastal areas, it was suggested that these tend to take up the share of the individual's antibody-producing resources that would otherwise be left idle in the absence of malaria.

6066

Curtain, C.C.; Kidson, C.; Champness, D.L.; Gorman, J.G. 1964. Malaria antibody content of gamma-2-7S globulin in tropical populations. Nature 203:1366-1377.

Detailed analyses of serum protein characteristics of Melanesian people in New Guinea have shown that the high gamma globulin levels observed are mainly the result of infections. Data accruing from comparisons of children with high and low rates of malarial infection, with respect to globulin levels and levels of malarial antibody, suggest that malaria may play a relatively minor part and that the total infective load may be more important in the etiology of the hypergammaglobulinemia. This conclusion is supported by the present quantitative data demonstrating the small contribution of malarial antibody total gamma-2-7S globulin and the absence of increase in malarial antibody gamma-2-7S globulin with increasing age. This is in contrast to the conclusions drawn by other workers, whose observations were made in Africa.

It is possible that the intense activity of the reticuloendothelial system in response to infections results in the production of nonspecific gamma globulin, including antibody directed toward autologous tissue determinants. The possibility that the production of autoantibodies may be one result of high rates of gamma globulin synthesis in tropical populations is worthy of further investigation.

6067

Diggs, C.L.; Sadun, E.H. 1965. Serological cross-reactivity between Plasmodium vivax and Plasmodium falciparum as determined by a modified fluorescent antibody test. Exp. Parasitol. 16:217-223.

The cross-reactivity between P. vivax and P. falciparum was studied by indirect fluorescent antibody. Two modifications were employed: use of liquid nitrogen for preservation of parasitized whole blood for the source of antigen, and use of Evans blue as a counterstain. Six of 29 sera from natural P. vivax infections reactive with P. vivax antigen were also reactive with P. falciparum antigen. Eleven of 21 serologically positive sera from natural P. falciparum infections were reactive with P. vivax antigen, two of these with the P. vivax antigen only. Sera from human volunteers with either P. vivax or P. falciparum infections were titrated in parallel tests with homologous and heterologous antigen. Geometrical mean reciprocal titers with P. falciparum sera were 28.3 for the homologous antigen and 6.3 for the heterologous antigen. For P. vivax sera the values were 17.2 and 9.3 for the homologous and heterologous antigens. Values for the P. falciparum sera were 132 with the homologous antigen and 20.0 with the heterologous antigen; the P. vivax sera gave values of 30.0 and 11.9 with the homologous and heterologous antigens. Two hundred and forty-six sera from subjects presumably free of malaria revealed 28 positives. There are both a species-specific and a group-specific component in these two organisms. The usefulness of the filter paper method of blood collection was confirmed.

6068

Gebbie, D.A.; Hamilton, P.; Hutt, M.; Marsden, P.; Voller, A.; Wilks, N. 1964. Malarial antibodies in idiopathic splenomegaly in Uganda. Lancet 2:392-393.

Of 40 patients with marked splenomegaly admitted to the Mulago Hospital, Kampala, 26 had a high malarial antibody titer and collections of lymphocytes in their hepatic sinusoids. The results suggest that idiopathic splenomegaly in Uganda is due to chronic malaria and that this can be diagnosed by liver biopsy.

6069

Ingram, R.L.; Carver, R.K. 1963. Malaria parasites: Fluorescent antibody technique for tissue stage study. Science 139:405-406.

Tissue stages of avian and simian malarias were stained by the fluorescent antibody method. The fluorescent bodies proved to be parasites in tests for immunological specificity and on restaining with Giemsa. These results suggest a method for studying two important aspects of mammalian malarias: the cycle in the mammalian host and the immunological relationships among malarias indicated by tissue stages.

6070

Kreier, J.P.; Ristic, M. 1964. Detection of a Plasmodium berghei - antibody complex formed in vivo. Amer. J. Trop. Med. Hyg. 13:6-10.

Direct staining of Plasmodium berghei with fluorescein-labeled antiserum from infected rodents indicated that these animals are capable of producing an antibody against the parasite. A naturally occurring P. berghei - antibody complex was detected by exposing parasitized erythrocytes from infected mice to fluorescein-labeled antimouse globulin.

6071

Kuvin, S.F.; Tobie, J.E.; Evans, C.B.; Coatney, G.R.; Contacos, P.G. 1963. Production of malarial antibody: Determination by the fluorescent-antibody technique. J. Amer. Med. Ass. 184:943-945.

The FA technique provides a specific and sensitive method for following the course of production of antibody in malaria and should prove to be a useful tool for the further study of immune mechanisms in this disease. The raised gamma globulin corresponds to increased anti-malaria antibody. The humoral part of malaria immunity is contained in the gamma globulin.

6072

Kuvin, S.F.; Voller, A. 1963. Malarial antibody titers of West Africans in Britain. Brit. Med. J. 1963:477-479.

Sera from 26 West Africans resident in Britain for 3 months to 7 years were examined for malarial antibody by FA. All the sera contained malarial antibody in low titer, suggesting that antibody production is reduced on leaving endemic malarial areas. This reinforces the clinical opinion that repeated infection with the malaria parasite is necessary to maintain adequate protective levels of malarial antibody. The simian parasite P.c. bastianellii demonstrates considerable cross-reactivity with human-malaria parasites and appears to be useful as an antigen that can be maintained in laboratory monkeys and used in diagnosis.

This method may prove to be a valuable tool in establishing a diagnosis of chronic or latent malaria and in the screening of blood donors for the presence of past or present infection with this disease. The combination of low malaria-antibody titers and high serum gamma globulin levels in West Africans resident in Britain suggests that malarial antibody is only a small fraction of the excess gamma globulin produced in tropical populations.

6073

McGregor, I.A.; Williams, K.; Voller, A.; Billewicz, W.Z. 1965. Immunofluorescence and the measurement of immune response to hyperendemic malaria. Trans. Roy. Soc. Trop. Med. Hyg. 59:395-414.

The results of an attempt to titer malarial antibodies in an African population are reported. Indirect FA was used. The subjects were of various ages and were in a hyperendemic environment. Fluorescent antibody titers were analyzed with respect to age groups, parasitemia, gametocytemia, spleen size, malnutrition, and other factors. Fluorescent antibody may fill the very real need for an accurate method of malarial antibody titration in the study of malarial epidemiology.

6074

Sodeman, W.A., Jr.; Jeffery, G.M. 1964. Immunofluorescent staining of sporozoites of Plasmodium gallinaceum. J. Parasitol. 50:477-478.

Sporozoites of P. gallinaceum were stained by direct FA. Immune serum was obtained from an infected chicken, and its preparation is described. Nonspecific background fluorescence of exoskeleton fragments was quenched by counterstaining with either Evans blue, RB 200-BSA, or Flazo orange. No sporozoite staining was seen using conjugated human antiserum against P. vivax, P. falciparum, or P. cynomolgi. Brightly fluorescing masses were seen on the tip end and lateral wall of some sporozoites. These areas appeared to correspond with the apical cup and the micropyle.

6075

Sodeman, W.A., Jr.; Jeffery, G. 1965. Immunofluorescent studies of Plasmodium berghei: A natural antibody in white mice. Amer. J. Trop. Med. Hyg. 14:187-190.

Pooled or individual sera from a portion of normal mice from a standard laboratory colony were shown to contain an antibody that reacted specifically with Plasmodium berghei antigen, as demonstrated by the indirect and direct fluorescent antibody methods. This antibody could be demonstrated in vitro when reacted with P. berghei antigen, then treated with FITC-conjugated antiserum, or as in vivo - bound antibody on antigen derived from P. berghei - infected mice. The source of antigen giving rise to this natural antibody has not been identified.

6076

Tobie, J.E. 1964. Detection of malaria antibodies: Immunodiagnosis. Amer. J. Trop. Med. Hyg. 13:195-203.

The serodiagnostic tests covered include precipitins, agglutination, hemagglutination, complement fixation, and fluorescent antibody tests. Considering the results obtained by the various methods, the complement fixation, the fluorescent antibody, and perhaps the hemagglutination reactions show the most promise for the detection of malaria antibodies. From a basic research standpoint, the complement fixation test has been most helpful and the fluorescent antibody technique, by virtue of its specificity and sensitivity, is beginning to assume an important role. These tests as practical aids in laboratory diagnosis await further development. As methods are refined and reagents standardized, it is believed that the fluorescent antibody technique will take a prominent position as an immunodiagnostic tool.

6077

Tobie, J.E.; Coatney, G.R. 1964. The antibody response in volunteers with cynomolgi malaria infections. Amer. J. Trop. Med. Hyg. 13:786-789.

The antibody response to the B strain in six patients and the M strain in one patient of Plasmodium cynomolgi was studied in volunteers with sporozoite-induced infections. In all cases malarial antibody was detected after, but not before, the initial parasitemia even throughout long prepatent periods, which in one individual extended 107 days. A comparison of the antibody response in patients with short or with long prepatent periods suggests that there is great similarity in respect to the abrupt rise in serum antibody levels after the initial parasitemia and to the subsequent course of antibody production.

6078

Tobie, J.E.; Kuvin, S.F.; Contacos, P.G.; Coatney, G.R.; Evans, C.B. 1963. Cross reactions in human and simian malaria. J. Amer. Med. Ass. 184:945-947.

Common antigens may be shared by P. cynomolgi and P. vivax parasites. P. cynomolgi may have an additional or perhaps different antigen or antigens that might account for the higher titers obtained. Both species in humans exhibit a tertian pattern of schizogony. Clinically, however, the disease caused by P. cynomolgi in man is generally less severe and of shorter duration than the infection produced by P. vivax.

6079

Voller, A.; Bray, R.S. 1963. Immunofluorescent staining of malaria parasites. Trans. Roy. Soc. Trop. Med. Hyg. 57:7.

Indirect FA staining was reported of trophozoites and gametocytes of Laverania falciparum and trophozoites of Plasmodium ovale and P. malariae, in thin blood smears, using immune African sera. The parasites were clearly fluorescent. In addition, fluorescent granules were obvious in the cytoplasm of the infected erythrocytes. Titers obtained using indirect FA as a measure of malarial antibody were given. Cord blood and serum from infants up to 2 weeks old gave high titers. Titers were low in older children up to an age of 2 years, after which the antibody level rose reaching a maximum in adults, especially after clinical malaria. FA staining was shown of erythrocytic stages of P. gallinaceum in thin blood smears and of exo-erythrocytic stages of P. gallinaceum in brain and liver impression smears.

6080

Voller, A.; Taffs, L.F. 1963. Fluorescent antibody staining of exo-erythrocytic stages of Plasmodium gallinaceum. Trans. Roy. Soc. Trop. Med. Hyg. 57:32-33.

The exo-erythrocytic stages of P. gallinaceum were stained in tissues by the direct fluorescent antibody method following a freeze-substitution procedure.

6081

Voller, A.; Wilson, H. 1964. Immunological aspects of a population under prophylaxis against malaria. Brit. Med. J. 2:551-552.

The study describes an investigation, in a hyperendemic falciparum malaria area, into the effects of antimalarial treatment on levels of circulating malaria antibody in a group of women and their children. Daraprim was used weekly to suppress malaria in the experimental group. The indirect fluorescent antibody test was used to measure levels of malarial antibody. None of the protected children had detectable malarial antibody, in contrast to the unprotected group, nearly all of whom had detectable antibody. The average titer of the protected mothers was much lower than that of the unprotected group. It is concluded that repeated and frequent infection is necessary to maintain malarial antibody at a high level.

E. TOXOPLASMA

6082

Andreoni, G.; Curatolo, D.; Rocchi, G.; Tosti, U.; Velli, V. 1965. Serological diagnosis of toxoplasmosis by the indirect immunofluorescence test. G. Mal Infett. 17:144-145. In Italian.

The authors tested 102 human sera by the technique of Sabin and Feldman and by indirect immunofluorescence. The test of Sabin and Feldman is more sensitive. The immunofluorescence test may constitute a useful collateral research tool in the serodiagnosis of toxoplasmosis.

6083

Camargo, M.E. 1964. Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. Rev. Inst. Med. Trop. Sao Paulo 6:117-118.

Minor technical modifications such as incubation of slides at 37 C for longer periods and use of conjugates devoid of nonspecific staining in lower dilutions, have led to results in the indirect fluorescent antibody test comparable to those of the Sabin-Feldman dye test. They were identical as to positivity and negativity and the titers were the same or differed at most by one dilution. In general, the fluorescence test has shown higher titers than the dye test.

6084

Dallenbach, F.; Piekarski, G. 1960. The nature of Toxoplasma gondii studied with the aid of fluorescein-labeled antibodies. Arch. Pathol. Anat. Physiol. 333:607-618. In German.

The Coons fluorescein-labeled antibody technique, direct and indirect methods, specifically stains toxoplasma in the spleen, lymph nodes, and liver of experimentally infected mice and guina pigs. The outer cell membrane of the organism fluoresces most intensely, probably indicating the site of antigen-antibody reaction. In acute stages of infection the organisms are demonstrable intact intra- and extra-cellularly. In chronic stages they lose sharply demarcated staining and may be recognized only in the liver and only by the presence of specific fluorescence. Intensity of the fluorescence is dependent on the titer of the serum used for staining, as measured by the Sabin-Feldman test. The higher the titer the more brilliant the fluorescence. The demonstration of toxoplasma by the Coons technique confirms the opinion that the Sabin-Feldman test has specific significance in evaluating the titer of antibody against toxoplasma.

6085

Fletcher, S. 1964. Serology of toxoplasmosis. Brit. Med. J. 2:1660.

This is a report of the routine use of indirect FA for the serologic diagnosis of toxoplasmosis. The methods are briefly described.

6086

Fletcher, S. 1965. Indirect fluorescent antibody technique in the serology of Toxoplasma gondii. J. Clin. Pathol. 18:193-199.

Indirect FA has been used to titrate human antibody against Toxoplasma gondii. Rigorous tests of the immunological validity of the method were made. The reactions of the gamma globulin of human immune serum with Toxoplasma gondii and fluorescent rabbit antihuman gamma globulin were thus specific. Thereafter the technique could be used with confidence to detect the reaction between doubling dilutions of human serum and smears of unfixed Toxoplasma gondii. In this way, titers were obtained with the indirect FA that agreed well with those of the dye test at both low and high levels of antibody. Compared with the dye test, the indirect FA has many advantages. The end-point is sharp and obviates the counting of stained and unstained organisms; supplies of antibody-free accessory factor sera are not needed. Prozones undetected by the dye test are strongly positive at screening dilutions. Reagents keep indefinitely and lend themselves to preparation, standardization, and issue by a central reference laboratory.

6087

Frezzotti, R.; Berengo, A. 1963. Identification of Toxoplasma gondii with fluorescein-labeled antibodies in the cerebrospinal fluid of patients with neuro-ophthalmic toxoplasmosis. Ophthalmologica Basel 145:72-76.

Preliminary observations on a method for the identification of Toxoplasma gondii with fluorescein-labeled antibodies in patients with neuro-ophthalmic toxoplasmosis are reported. The toxoplasma-like bodies that have been found in the cerebrospinal fluid sediment of these patients are fluorescein-positive and can be considered as actual toxoplasmas. The direct identification test on cerebrospinal fluid and the specific immunofluorescence reaction provide thus an etiological diagnosis, clinically available, that was previously impossible in ocular toxoplasmosis.

6088

Fulton, J.D.; Voller, A. 1964. Evaluation of immunofluorescent and direct agglutination methods for detection of specific toxoplasma antibodies. Brit. Med. J. 2:1173-1175.

Toxoplasma antibodies in human sera have been quantitatively assessed by indirect FA. The results obtained correlate well with those obtained by the dye test, by the direct agglutination test, and by CF. The FA method is slower, but its specificity has been established by absorption studies and by lack of cross-reaction with sera from cases of trypanosomiasis, malaria, kala-azar, sarcosporidiosis, leptospirosis, syphilis, schistosomiasis, and filariasis.

6089

Garin, J.-P.; Ambroise-Thomas, P. 1963. Serological diagnosis of toxoplasmosis with the indirect fluorescent antibody method. Presse Med. 71:2485-2488. In French.

In an indirect two-layer method, 189 sera were studied and the results were compared with those of the Sabin-Feldman and the complement fixation tests. A strict agreement was found in 174 samples. The technical difficulties of this reaction do not as yet permit its routine use for serological diagnosis and the toxoplasma lysis test is not yet obsolete.

6090

Kramar, J. 1964. The use of fluorescent antibodies in serological diagnostics of toxoplasmosis. Cesk. Parasitol. 11:159-164. In Czech.

The indirect method and the inhibition reaction using FA were applied to titer antitoxoplasmic antibodies in human sera. Preparation of fluorescent antibodies with FITC is described. In sera examined by the indirect method the determined qualitative differences in the intensity of fluorescence were more exact than in the inhibition reaction. The inhibition reaction is more suitable for differentiation of negative and very positive sera, but, when used on sera with low titer, the results may not be exact. Both methods were compared with the complement-fixation reaction.

6091

Kramar, J.; Cerna, Z.; Chalupsky, J. 1964. Immunofluorescence reactions in the serological diagnosis of toxoplasmosis. Zentralbl. Bakteriol. 193:523-534. In German.

One hundred and twenty-seven human sera were investigated quantitatively for the presence of Toxoplasma antibodies with the aid of the indirect

fluorescence antibody reaction (IFAR) and the fluorescence inhibition reaction (FIR). The results with both reactions were compared with those using the CF test. With the help of the IFAR the presence of antibodies was demonstrated in the great majority of cases in higher titers than in the CF test and the FIR. The FIR showed itself to be less sensitive than the IFAR and comparatively also less sensitive than the CF test. There were frequent discrepancies between the IFAR and the FIR. The IFAR is recommended as very suitable for the quantitative estimation of Toxoplasma antibodies, and the FIR as a method for checking the IFAR, above all in the preliminary assessment of sera, and for differentiating strongly positive sera from negative ones. It is proposed that with the IFAR, a titer value of 1:10, 1:8, or more is taken as evidence of positivity.

6092

Kramarzh, Y. 1963. Fluorescent antibodies in the serological diagnosis of toxoplasmosis. Med. Parazitol. 32:454-460.

The discussion covers the use of FA to detect antitoxoplasmosis antibodies in human sera by the indirect method. A comparison of the results obtained through the use of the indirect method with those of the complement fixation test demonstrated that sera appearing negative in the complement fixation test appear negative or mildly positive by the indirect method. Sera that in the CFT show titers of 1:10 and 1:20 with the indirect method respond either mildly positive or positive. Sera with the CFT titers of 1:40 and over furnish a clear-cut positive response.

6093

Labzoffsky, N.A.; Fish, N.A.; Gyulai, E.; Roughley, F. 1965. A survey of toxoplasmosis among mentally retarded children. Can. Med. Ass. J. 92:1026-1028.

To determine what role, if any, toxoplasmosis plays in the mental retardation of children, sera from 345 mentally retarded children were tested for the presence of antibodies to Toxoplasma gondii. The serological tests employed were the complement fixation, the Sabin-Feldman dye test, and the immunofluorescence test. Of 345 mentally retarded donors, nine gave a positive skin reaction, 15 had complement-fixing antibodies, 21 had immunofluorescence antibodies, and 45 had dye test antibodies to T. gondii. The incidence of antibodies to T. gondii in the mentally retarded group was approximately the same as in the normal control group and less than in the groups suspected of having toxoplasmosis. Toxoplasmosis played little or no role as a predisposing factor in the occurrence of congenital mental deficiency.

6094

Naumann, G.; Wildfuhr, G. 1965. The significance of immunofluorescence for serodiagnosis of infectious diseases. Munchen Med. Wochensch. 107:1384-1386. In German.

The author discusses the results obtained by the immunofluorescence method in serodiagnosis of various infectious diseases. Comparative studies with standard methods show that this method is of greater significance in serological diagnosis of syphilis, toxoplasmosis, pseudotuberculosis, cryptococcosis, and trichinellosis.

6095

Robertson, A.; Appel, M.; Ruckerbauer, G.M.; Bannister, G.L.; Boulanger, P. 1963. Toxoplasmosis: III. Studies using the complement-fixation test and fluorescence-inhibition test with sera of experimentally exposed birds. Can. J. Comp. Med. Vet. Sci. 27:189-192.

The direct, modified direct, and indirect complement-fixation (CF) tests and the fluorescence-inhibition (FI) test were investigated using sera from pigeons, chickens, and turkeys that had been exposed to Toxoplasma gondii. The direct CF test was suitable for use with pigeon sera. The indirect CF method effectively demonstrated antibodies in chicken and turkey sera. FI tests were less sensitive than the CF methods.

6096

Ruckerbauer, G.M.; Robertson, A.; Bannister, G.L.; Boulanger, P.; Beauregard, M. 1963. Toxoplasmosis: I. Studies by the fluorescein-labelled antibody technique. Can. J. Comp. Med. Vet. Sci. 27:27-33.

FA was investigated for the diagnosis of toxoplasmosis. The direct method, the inhibition and indirect modifications are suitable for the demonstration of Toxoplasma gondii in fluid and tissue-impression slides from animals in the acute phase of infection. The method was not applicable to frozen tissue sections. The inhibition technique detected antibodies in immune sera from various species of animals. However, the titers obtained were lower than with the complement-fixation test.

6097

Stadtsbaeder, S.; Tellier-Verheyden, N.; Weber, M. 1964. Serologic diagnosis of toxoplasmosis by immunofluorescence. Acta Clin. Belg. 19:161-166. In French.

An immunofluorescent method is described for the serologic diagnosis of toxoplasmosis. The first results obtained can be compared with those yielded by the classic reaction of toxoplasmolysis or Sabin Feldman dye test.

6098

Tsunematsu, Y.; Shiori, K.; Kusano, N. 1964. Three cases of lymphadenopathia toxoplasmotica: With special reference to the application of fluorescent antibody technique for detection of Toxoplasma in tissue. Jap. J. Exp. Med. 34:217-230.

Three cases of lymphadenopathia toxoplasmotica, first found in Japan, are presented. In two of these three cases, the presence of Toxoplasma in the lymph nodes was demonstrated by the direct fluorescent antibody technique. The results of serologic test, fluorescent antibody staining, histological examination, and mouse inoculation are discussed in respect to their significance in the diagnosis of toxoplasmosis.

6099

van Nunen, M.C.J.; van der Veen, J. 1965. Examination for toxoplasmosis by the fluorescent antibody technique. Trop. Geogr. Med. 17:246-253.

An investigation was carried out into the usefulness of indirect FA in the serological diagnosis of toxoplasmosis. Brains of mice infected intracerebrally with toxoplasma served as the antigen. The reproducibility of the test was similar to that of the dye test of Sabin and Feldman. In most instances the titers by indirect FA were identical with those of the Sabin-Feldman reaction or slightly lower. In rabbits infected experimentally with toxoplasma there was no difference between the two reactions in the time of appearance or the level of antibody. Indirect FA is recommended for use in the diagnosis of toxoplasmosis.

6100

van Nunen, M.C.J.; van der Veen, J. 1965. Examination for toxoplasmosis by the fluorescent antibody technique. Ned. Tijdschr. Geneesk. 109:742-746. In Dutch.

A study was made of the applicability of the indirect fluorescent antibody technique for the serodiagnosis of toxoplasmosis. Brains of mice infected intracerebrally with Toxoplasma were used as an antigen. The reproducibility of the test was similar to that of Sabin and Feldman's dye test. In most cases the titers of the indirect fluorescent antibody test were similar to those of the dye test, or slightly lower. In rabbits experimentally infected with Toxoplasma, no difference in time of appearance and antibody level was seen between the two tests. The indirect fluorescent antibody technique is recommended for use in the diagnosis of toxoplasmosis.

6101

Zardi, C. 1963. Fluorescent antibodies in the diagnosis of toxoplasmosis. Nuovi. Ann. Ig. Microbiol. 14:585-612. In Italian.

Experiments have been made on the utility of fluorescent antibodies. This has already been demonstrated in studies of various immunohistochemical problems, specifically those concerning the morphological localization of the antigens in the tissue, on viruses, bacteria, Rickettsia and Protozoa, with highly significant results. On the basis of parallel research made with other serodiagnoses, serodiagnosis with fluorescent antibodies for toxoplasmosis can be considered a highly specific and sensitive test.

F. TRYPANOSOMA

6102

Beck, J.S.; Walker, P.J. 1964. Antigenicity of trypanosome nuclei: Evidence that DNA is not coupled to histone in these protozoa. Nature 204:194-195.

All human sera stained the kinetoplast area in all species of trypanosomes investigated, but markedly brighter staining in kinetoplastic forms was produced with sera containing anti-DNA antinuclear antibody. In most species the stained area corresponded in size, shape, and position to the kinetoplast, but a similar area of staining was seen in the strain T. evansi S.A.K., in which neither kinetoplasts nor their DNA could be demonstrated with the light microscope. Electron microscope investigations have shown that the kinetoplast is a modified mitochondrion containing a band of DNA and that similar structures, lacking only the DNA band, are seen in drug-induced and spontaneously occurring akinetoplastic trypanosomes. In the nonspecific staining of the kinetoplast region produced by human serum, the human gamma globulin is attached to the kinetoplast at some point other than in the DNA band, although the resolution of the optical system used in this investigation is such that it is not possible to localize the staining with great accuracy to the kinetoplast. Alternatively, it is possible that this staining has resulted from trapping of human serum in the invagination of the cell membrane at the base of the flagellum.

6103

Essenfeld, E.; Fennell, R.H., Jr. 1964. Immunofluorescent study of experimental Trypanosoma cruzi infection. Proc. Soc. Exp. Biol. Med. 116:728-730.

Guinea pigs were infected with Trypanosoma cruzi. Five different strains were used and cross-immunity was evident among these strains. Serum from convalescent guinea pigs conjugated with fluorescein could be used

as a stain for both the blood trypanosomal form and the tissue leishmanial form. Antibodies against the forms could also be identified by the indirect technique.

6104

Gil, B.S. 1965. Studies on the serological diagnosis of Trypanosoma evansi. J. Comp. Pathol. 75:175-183.

The efficacy of various immunological techniques to detect antibodies to T. evansi has been compared. The agglutination and indirect hemagglutination test proved most sensitive as they detected infection in 5 days and gave high titers. The precipitin and FA tests became positive within 20 days. The CF test proved least sensitive. The agglutination tests proved most sensitive. Cross-reactions with the serological variants were complete but further work is needed with more strains. The indirect hemagglutination, FA, and CF tests gave complete cross-reaction with heterologous reagents. The antigen seemed common to all populations of T. evansi.

6105

Lucasse, C. 1964. Fluorescent antibody test as applied to cerebrospinal fluid in human sleeping sickness. Bull. Soc. Pathol. Exot. 57:283-292.

FA was used to test the cerebrospinal fluid (CSF) of 29 patients and two chimpanzees infected with Trypanosoma gambiense and one chimpanzee infected with T. rhodesiense (including five human samples preserved at 2 C for 10 months), and of six uninfected persons. It was also used on the sera of these patients. The test was very sensitive, the dilution titers and intensity of fluorescence paralleling the intensity of cell and protein alterations. Negative CSF samples and sera did not stain. BA-46-76543.

6106

Romana, C. 1964. Adaptation of the fluorescent antibody technique (inhibition test of Goldman) to the diagnosis of Chagas's disease. Acta Physiol. Lat. Amer. 14:401.

FA was adapted to the diagnosis of Chagas's disease. Sixty-three sera were tested, 35 positive for T. cruzi infections, and 28 normal sera or positive to toxoplasmosis. There was 96 per cent agreement of FA and complement fixation for T. cruzi. Serologic findings indicate that FA can play an important role in Chagas's disease serodiagnosis.

6107

Sadun, E.H.; Duxbury, R.E.; Williams, J.S.; Anderson, R.I. 1963. Fluorescent antibody test for the serodiagnosis of African and American trypanosomiasis in man. J. Parasitol. 49:385-388.

A fluorescent antibody technique for the serodiagnosis of African and American trypanosomiasis in man is described. The test can be performed simply and rapidly through the use of trypanosomes in blood smears as antigen. There were extensive cross-reactions with sera from patients with other species of trypanosomes. Tests of sera from healthy controls and from patients with nontrypanosomal diseases and disorders revealed relatively few cross-reactions, indicating a high degree of specificity. The finding that dried blood on absorbent paper could be tested successfully with the FA technique may indicate its usefulness for studies in endemic areas.

6108

Shaw, J.J.; Voller, A. 1964. The detection of circulating antibody to kala-azar by means of immunofluorescent techniques. Trans. Roy. Soc. Trop. Med. Hyg. 58:349-352.

Circulating antibody was detected by FA. This technique is worthy of further consideration in the diagnosis and study of visceral leishmaniasis. It is clear that this test is, as yet, only group-specific, but this is not a serious limitation except in regions of the world where both trypanosomiasis and leishmaniasis are endemic. Discussion of other tests and implications is included.

6109

Toussaint, A.J.; Tarrant, C.J.; Anderson, R.I. 1965. An indirect fluorescent antibody technique using soluble antigens for serodiagnosis of Trypanosoma cruzi infection. Proc. Soc. Exp. Biol. Med. 120:783-785.

A soluble antigen fluorescent antibody (SAFA) technique for the serorecognition of American trypanosomiasis is described. The technique has the following advantages over the conventional whole-organism indirect fluorescent antibody tests: It permits the investigator to select the antigen objectively; it provides for mechanical reading of test results; it eliminates the problem of fading; and it accounts for nonspecific fluorescence contributed by the serum of the patient, e.g., drugs and/or free fluorescein in the conjugated antiserum. Preliminary investigations revealed that exosomatic carbohydrate and somatic protein antigens from T. cruzi would adhere to the artificial matrix. The sensitivity and specificity of the somatic protein and exoantigens were evaluated. The findings indicate that the SAFA technique with either of these two antigens should yield excellent results.

6110

Toussaint, A.J.; Tarrant, C.J.; Anderson, R.I. 1965. Soluble antigen fluorescent antibody (SAFA) test for the serorecognition of infection with Trypanosoma cruzi. J. Parasitol. 51(Suppl.):29.

An SAFA technique was developed and evaluated with appropriate antisera and a soluble somatic protein antigen obtained from Schistosoma mansoni cercariae. This procedure permits objective selection of antigen and allows results to be read by precise fluorometric methods. The current investigations were initiated to determine the potential of the SAFA technique in Chagas' disease and to ascertain whether antigens in addition to somatic proteins could be employed. Exoantigen (glycoprotein), carbohydrate, and protein somatic antigens from T. cruzi were studied. These antigens could be employed without modifying the SAFA technique. Only the somatic protein and exoantigens were evaluated since the carbohydrate antigen had shown the least satisfactory results in CF tests. The SAFA technique readily differentiated between individuals with Chagas' disease and uninfected persons. Occasional borderline reactions were encountered. The SAFA test is somewhat more sensitive and at least as specific as CF procedures for American trypanosomiasis. Ability to employ various antigens in the technique suggests possible application of the method for serodiagnosis of many infectious diseases. Complete article.

6111

Voller, A. 1963. Immunofluorescent observations on Trypanosoma cruzi. Trans. Roy. Soc. Trop. Med. Hyg. 57:232.

T. cruzi was FA stained. The slide antigen was acetone-fixed smears. Both blood and culture forms fluoresced especially strongly in the region of the kinetoplast and around the nucleus, the central part of which did not stain. Heterologous conjugates from antisera to plasmodia or helminths did not react with T. cruzi. When excess unlabeled serum was mixed with the homologous conjugate, the fluorescent staining of T. cruzi was abolished, owing to competition for antigen sites. When normal non-immune serum was mixed with the conjugate, the reaction was not markedly diminished. This one step inhibition procedure may possibly be of use as a screening method to detect the presence of antibodies, to T. cruzi, in human serum. Complete article.

6112

Weitz, B. 1963. The specificity of trypanosomal antigens by immunofluorescence. J. Gen. Microbiol. 32:145-149.

The immunofluorescent method was applied to a strain of Trypanosoma brucei and of Trypanosoma vivax by using direct FA. Conjugated antibodies to the soluble antigens of trypanosomes reacted specifically with the homologous species only; antibodies to the bound antigens reacted with both species.

6113

Williams, J.S.; Duxbury, R.E.; Anderson, R.I.; Sadun, E.H. 1963. Fluorescent antibody reactions in Trypanosoma rhodesiense and T. gambiense in experimental animals. J. Parasitol. 49:380-384.

A fluorescent antibody technique for the serodiagnosis of African trypanosomiasis in experimental animals is described. The indirect fluorescent antibody test employing the trypanosome form from rats as antigen appears to be highly sensitive in rabbits infected with T. rhodesiense and T. gambiense. Results of 29 sera from rabbits infected with T. rhodesiense organisms yielded 29 positive reactions; 21 positive reactions and one weak reaction were obtained from 22 rabbits infected with T. gambiense organisms. The reproducibility of this test employing different lots of trypanosomes and labeled antiglobulin was good. Antibodies were detected between 1 and 2 weeks after inoculation of animals, with no apparent correlation between the early appearance of antibodies and the size of the inoculum. The similarity of observable reactions between the African trypanosomes and T. lewisi as antigen is of great interest.

G. OTHER PROTOZOA

6114

Andersen, F.L. 1963. The site of the immune reaction against Eimeria bovis in calves and the demonstration of antibodies by immunofluorescence. Diss. Abstr. 24:3020.

In eleven experiments 4 to 6 of 7 to 12 calves were immunized with 25,000 to 100,000 Eimeria bovis oocysts, and in three experiments 8 of 13 calves were immunized with 0.5 or 1.0 million oocysts. Approximately one month later these calves and the remaining uninoculated calves were challenged. Treatment results are given. Clinical and histologic findings are discussed. In two experiments blood samples were collected at frequent intervals. The sera were titered by the indirect FA. Merozoites were used as antigen and were obtained by crushing fresh schizonts on clean glass slides. A ring of fluorescence around the merozoites indicated antibody in serum. Fluorescence of the merozoites was not observed with serum collected from any of the calves until 10 to 22 days after inoculation. After that time serum from each calf was positive. The calves that received only one large inoculation attained high titers on the average in less time than did the calves that were inoculated twice.

6115

Andersen, F.L.; Lowder, L.J.; Hammond, D.M.; Carter, P.B. 1965. Antibody production in experimental Eimeria bovis infections in calves. Exp. Parasitol. 16:23-35.

Two experiments were conducted to investigate the possible presence of humoral antibodies in calves experimentally infected with Eimeria bovis. Ten calves received 25,000 oocysts and 1 million oocysts 26 or 27 days later; eight calves were inoculated once with 1 million oocysts. Blood samples were taken periodically and the sera collected were tested against first-generation merozoites by indirect FA and the slide agglutination test, and against an oocyst extract by the precipitin ring test. In vitro observations were also made on the effect of immune serum upon the first-generation merozoites. Fluorescence of the merozoites was first observed in serum collected 10 to 22 days after inoculation. The calves that received only one large inoculum attained, on the average, higher titers in less time than did the calves inoculated twice. Agglutinating antibody was first detected 7 to 17 days after inoculation; however, in this case calves that received a second inoculum developed higher titers than did those inoculated once. The results of the precipitin ring tests paralleled closely those of the slide agglutination tests.

6116

Barrow, J.H., Jr.; Miller, H.C. 1964. Fluorescence of an antibody specific for Leucocytozoon in the globulins of egg white. J. Parasitol. 50(Suppl.):45.

Egg white was isolated from eggs laid by Peking and mallard ducks with known history of Leucocytozoon (positive egg products) and from known uninfected duck eggs (negative egg products). The whites were removed under aseptic technique, treated with 0.01 per cent merthiolate, and frozen until used. One beta and two gamma globulins were precipitated from the egg whites by dialysis in distilled water. The globulins (0.5 ml in 15 ml 0.85 per cent NaCl) were conjugated with fluorescein isothiocyanate. A fluorescence microscope was used for both study and photographs of the reactions. Antibody specificity was demonstrated by both selective staining of the parasites on blood films and the blockage of the reaction after the pretreatment of the slides with unlabeled antibody. The negative egg products failed to block the reaction. The egg products from ducks previously infected with Leucocytozoon reacted with Leucocytozoon only; those positive for Leucocytozoon and Haemoproteus reacted with these two genera and Plasmodium. Complete article.

6117

Brzosko, W.; Nowoslawski, A. 1963. Immunohistochemical studies on Pneumocystis pneumonia. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 11:563-564.

Immunoelectrophoretically monospecific, FITC labeled globulin fractions (albumin, gamma globulin, and fibrinogen) were used. Large quantities of gamma globulin exudate were found closely bound to Pneumocystis carinii conglomerates in the bronchiolar and alveolar lumina; prolonged washing of sections in a 0.02 M citrate buffer solution at pH 3.3 resulted in almost complete removal of intraalveolar gamma globulin from the P. carinii conglomerates. Small amounts of albumin were detected in the alveolar exudate, but unlike the gamma globulin, it was quickly washed out with physiological saline. Sections of lung stained for fibrin and/or fibrinogen revealed specific fluorescence in the vascular lumina only; no fibrin and/or fibrinogen was demonstrated in the intra-alveolar exudate. There was a marked activation of germinal centers in all of the peribronchial and hilar lymph nodes with the presence of many mature and Russell-body plasma cells. BA-46-13333.

6118

Goetz, O. 1965. Fluorescence microscopic studies in interstitial plasma cell pneumonia. Monatsschr. Kinderheilk. 113:194-197. In German.

These tests are a further contribution to the etiological importance of pneumocysts in interstitial plasmacellular pneumonia.

6119

Kreier, J.P.; Ristic, M. 1963. Morphologic, antigenic, and pathogenic characteristics of Eperythrozoon ovis and Eperythrozoon wenyoni. Amer. J. Vet. Res. 24:488-500.

Eperythrozoon ovis and Eperythrozoon wenyoni were studied by Giemsa and fluorescein-labeled antibody staining, phase-contrast microscopy, complement fixation tests, and by electron microscopy, using shadow-cast and ultra-thin section techniques. The organisms were antigenically similar. Stained by the Giemsa technique, E. ovis had a higher stain affinity for basophilic dye than did E. wenyoni, which appeared most characteristically as a delicate ring structure. E. ovis more frequently appeared as a solid disk or rod located on the surface at the periphery of the erythrocyte. In wet preparations observed by phase contrast, eperythrozoa appeared as spheres. Eperythrozoa were stained with FA obtained from hyperimmunized Eperythrozoon carriers. The fluorescent bodies were morphologically similar to those seen in Giemsa-stained blood films. Serum from animals infected with E. ovis and E. wenyoni reacted with Anaplasma marginale antigen in complement-fixation tests. Electron microscopic findings are described, and clinical findings are compared.

6120

Ristic, M.; Oppermann, J.; Sibinovic, S.; Phillips, T. 1964. Equine piroplasmosis - a mixed strain of Piroplasma caballi and Piroplasma equi isolated in Florida and studied by the fluorescent antibody technique. Amer. J. Vet. Res. 25:15-23.

The globulin fraction was separated from the serum of a horse experimentally infected with Piroplasma parasite (Florida isolate) and conjugated to fluorescein isothiocyanate. Alcohol-fixed organisms in the blood films of infected splenectomized horses manifested fluorescence when brought in contact with the conjugated globulin. On the basis of size, mode of multiplication, and number of parasites in infected erythrocytes, it was concluded that the infectious blood sample from Florida contained both Piroplasma caballi and Piroplasma equi. Further comparisons, by immunoserologic techniques, between these species and one or more strains and species for each of these Piroplasma are needed in order to identify the organisms in the Florida material. The immunologic specificity of the fluorescent staining was established by use of proper control procedures.

6121

Ristic, M.; Sibinovic, S. 1964. Equine babesiosis: Diagnosis by a precipitation in gel and by a one-step fluorescent antibody inhibition test. Amer. J. Vet. Res. 25:1519-1526.

By means of precipitation with protamine sulfate, a soluble antigen was obtained from erythrocytes of horses acutely infected with Babesia caballi. A precipitating antibody was demonstrated in horses with babesiosis by bringing the soluble Babesia antigen in gel into contact with sera of affected horses. Acute or subclinical latent-phase sera gave equal precipitation responses. The specificity of the test was shown by the absence of reaction with sera of horses with various other infections, including viral infectious anemia. A one-step fluorescein-labeled antibody inhibition test was found useful in a preliminary study to detect Babesia carrier horses. In its present state, this test appears more suitable as a research tool than for routine diagnosis.

6122

Schaeffler, W.F. 1963. Serologic tests for Theileria cervi in white-tailed deer and for other species of Theileria in cattle and sheep. Amer. J. Vet. Res. 24:784-791.

A capillary agglutination test was developed for Theileria using an antigen prepared by sonicating erythrocytes from white-tailed deer (Dama virginiana) infected with Theileria cervi. Antibodies against this antigen were found in the serum of deer experimentally infected with T. cervi, in the serum of cattle experimentally infected with T. parva, T. annulata, or T. lawrencei,

and in the serum of sheep experimentally infected with T. recondita. Serum samples of 115 deer killed by hunters in southern Illinois were tested by the capillary agglutination test. Four were positive, and T. cervi was found in blood smears from two of them. Serum taken from animals following the acute stage of infection seemed to react more strongly than serum taken from animals before or during the peak of the acute reaction. Circulating antibodies could not be detected by the FA technique or the gel-diffusion test in infected calves or deer, but they were found in serum from rabbits hyperimmunized against T. cervi.

6123

Sharma, N.N.; Foster, J.W. 1964. Serology of Eimeria tenella oocysts with rabbit serum. J. Parasitol. 49:943-946.

The antigenicity of Eimeria tenella preparations was studied by capillary-tube flocculation and indirect fluorescent antibody tests. Oocyst extracts reacted weakly and somewhat specifically in flocculation tests. Reactions of oocyst walls, sporocysts, and sporozoites were as great with normal serum as with antiserum. Marked fluorescence was seen in oocyst walls tested by the indirect fluorescent antibody test, but sporocyst walls fluoresced weakly. Nonspecific reaction was much reduced by absorption with liver powder and by dilution, but was not completely eliminated.

6124

Zaman, V. 1965. The application of fluorescent antibody test to Balantidium coli. Trans. Roy. Soc. Trop. Med. Hyg. 59:80-82.

The fluorescent antibody test on Balantidium coli is described. Experience with unfixed and fixed specimens has shown that there is no nonspecific staining with the unfixed specimens; the controls remain completely negative. With the fixed specimens the controls show a certain amount of nonspecific fluorescence. It is, however, possible to differentiate the test slides from the controls by the difference in the intensity of the fluorescence. In terms of brightness, acetone gave the best results among the five different fixatives tried.

IV. RICKETTSIAE

6125

Alkan, W.J.; Evenchik, Z.; Eshchar, J. 1965. Q fever and infectious hepatitis. Amer. J. Med. 38:54-61.

Twenty-four cases of Q fever hepatitis are reported. The diagnosis was confirmed by serological examinations including FA. Specific urinary antigen was demonstrated. Clinically, the disease is almost indistinguishable from infectious hepatitis. Epidemiological and statistical evidence support the contention that Q fever hepatitis is a separate disease entity. The delineation of Q fever hepatitis and its distinction from infectious hepatitis helps to clarify the multicausal origin of virus hepatitis.

6126

Balaeva, N.M.; Korn, M.Ya.; Kulberg, A.Ya. 1963. Detection of antibodies to Rickettsia prowazeki by the fluorescent serological method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:1:52-57. In Russian.

This work was undertaken to elaborate optimal conditions for the detection of antibodies to Rickettsia prowazeki in typhus patients with the aid of the fluorescent serological method. The specificity of this reaction was studied by comparing its results with those of the complement fixation test and neutralization reaction of the toxic substance of rickettsiae. The significance of the pH of physiological saline used for sera dilution, as well as the effect of serum inactivation and of the methods of rickettsia antigen fixation on a slide, was ascertained.

6127

Balaeva, N.M.; Levina, Ye.N. 1964. Detection of Rickettsia prowazekii. Izv. Akad. Nauk SSSR Ser. Biol. 3:433-438. In Russian.

The investigations testify to the feasibility of detecting Rickettsia prowazekii by direct FA procedures. They may be detected in smears from the lungs of white mice and in the intestines of clothes lice infected with these rickettsiae. The feasibility of detecting the various forms of the causative agent during its development was demonstrated on a model of a Rickettsia prowazekii culture.

6128

Balaeva, N.M.; Levina, E.N.; Korn, M.Ya. 1965. Fluorescent antibodies for Rickettsia detection. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:3:17-21. In Russian.

The authors discuss literature data and personal experience in use of fluorescent antibodies for the detection and identification of Rickettsia. The special significance of this method in mass examination of carriers for natural Rickettsia infection is emphasized.

6129

Bozeman, F.M.; Elisberg, B.L. 1963. Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc. Soc. Exp. Biol. Med. 112:568-573.

The indirect fluorescent antibody test, in which dilutions of sera from patients with scrub typhus were tested on smears of Rickettsia tsutsugamushi, gave results that were serologically diagnostic of the disease in each instance. Nonspecific reactions did not occur when the scrub typhus sera were exposed to antigens of other species of Rickettsia. Likewise, there was no reaction between R. tsutsugamushi smears and sera from other rickettsial infections, or with sera from patients with Proteus OXK agglutinins of non-rickettsial origin with one exception, i.e., a case of leptospirosis from Puerto Rico.

6130

Burgdorfer, W. 1963. Investigation of 'transovarial transmission' of Rickettsia rickettsii in the wood tick, Dermacentor andersoni. Exp. Parasitol. 14:152-159.

In a quantitative study of transovarial passage of Rickettsia rickettsii to the progeny of experimentally and naturally infected wood ticks, Dermacentor andersoni, it was found that all infected female ticks tested passed rickettsiae via eggs to almost 100 per cent of their offspring. Infection was retained throughout the filial generation and was again transmitted by all F-1 females tested to 100 per cent of their progeny. Examination of FA stained sections of ovarian tissues of infected female ticks revealed the presence of rickettsiae in every ovum; organisms were regularly found in the cytoplasm but never in the nuclei of developing eggs. During the study, infectivity of the strains of R. rickettsii remained unchanged, as indicated by inoculation of tick eggs into chick embryos and by feeding of ticks on guinea pigs.

6131

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. Virology 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

6132

Iida, T.; Kawashima, H.; Kawamura, A. 1965. Direct immunofluorescence for typing of tsutsugamushi disease rickettsiae. J. Immunol. 95:1129-1133.

Fluorescent antibody prepared from sera from guinea pigs immunized with Gilliam, Karp, and Kato strains of tsutsugamushi disease rickettsiae showed higher staining titers against the homologous than against the heterologous strains. A possible application of the direct immunofluorescent technique for typing of newly isolated rickettsia strains is suggested.

6133

Krasnik, F.I. 1963. Demonstration of Rickettsia prowazekii in cell cultures by the fluorescent antibody method. Acta Virol. 7:190.

A combination of tissue culture and fluorescent antibody methods could become a valuable tool in early and rapid diagnosis of rickettsial infections.

6134

Krasnik, F.I. 1963. Use of the fluorescent antibody method for the study of Rickettsia prowazekii in tissue culture. Tr. Inst. Epidemiol. Mikrobiol. Gig. Leningrad 25:26-31. In Russian.

Application of the fluorescent antibody method to study of Rickettsia prowazekii in tissue cultures is very promising. By this method it was possible to observe the nature and intensity of multiplication of the causative agent dynamically, its localization, structure, and interrelationships with cells of various tissues. These preliminary data indicate that during the cultivation of rickettsiae in tissues the use of FA allows a more detailed study of the interrelationship between the causative agent and the cell. Along with this, further investigations in this direction may be promising for early and rapid detection of the causative agent during rickettsial infections.

6135

Krieg, A. 1964. Immunofluorescence-microscopic studies in relation to rickettsiosis of Melolontha spp. Z. Naturforsch. 19b:6:48-490. In German.

FA studies on the rickettsioses of Melolontha spp. showed that the shell of the accompanying protein crystals is antigenically related to the normal surface antigen of rickettsiae.

6136

Kundin, W.D.; Liu, C.; Harmon, P. 1964. Scrub typhus in mice: Fluorescent antibody studies. Bacteriol. Proc. M214:85.

The pathogenesis of the Karp strain of Rickettsia tsutsugamushi was studied by immunofluorescence. Unirradiated mice and mice X-irradiated at 400 r were inoculated intracerebrally (IC), intraperitioneally (IP), intramuscularly (IM), or subcutaneously (SC) with 1,000 mouse IP mean lethal doses and observed for 28 days. All irradiated mice died, as did unirradiated IC and IP mice. Two-thirds of the unirradiated IM and SC mice survived. Mice from all groups were sacrificed periodically. Antigenic involvement was studied by direct FA staining of thin sections cut from frozen whole torsos. In IC mice, fluorescence was generally observed only in the leptomeninges. In IP mice, antigen was observed in capsules of the kidneys, liver, and spleen, in intestinal serosa, connective tissue, and peritoneal exudate. Radiation accelerated and intensified antigenic involvement and hastened death in both groups. Only 3 of 12 IM or SC mice showed rickettsial antigen in tissues. If they were preirradiated, positive results increased to 10 of 17 examined. When fluorescence was observed, antigen was generally located in kidney, spleen, liver, lung, lymphatics, capsules, leptomeninges, connective tissue, and in skeletal muscle at the site of inoculation. Antigenic involvement was thus profoundly influenced by X-irradiation and by the route of inoculation.

6137

Kundin, W.D.; Liu, C.; Harmon, P.; Rodina, P. 1964. Pathogenesis of scrub typhus infection, Rickettsia tsutsugamushi, as studied by immunofluorescence. J. Immunol. 93:772-781.

The pathogenesis of R. tsutsugamushi in infected suckling and weanling mice was studied with fluorescein-labeled antibodies. Although the areas of involvement differed with the route of inoculation, rickettsial antigen was observed primarily in connective tissues of mesenchymal origin. X-irradiation increased the mortality, shortened the incubation period, and intensified the antigenic involvement. Rickettsiae could be found by infectivity titrations but not by fluorescent antibody staining in mice infected up to a year before. Attempts to cause a recrudescence by various stresses were unsuccessful.

6138

Lesso, J.; Brezina, R. 1964. Demonstration of Coxiella burneti in mice and guinea pigs by the fluorescent antibody method. Cesk. Epidemiol. 13:351-357. In Czech.

The potentialities of rickettsia detection by the fluorescent antibody, titration, and tinctorial methods in mice and guinea pigs infected with a highly virulent and a weakly virulent Coxiella burneti strain were studied. In the detection of Coxiella burneti by the fluorescent antibody method an important role is played by the virulence of the strain. This method was more sensitive than the tinctorial one as regards Coxiella demonstration. The difference was marked in demonstrating coxiellae in mice infected with the weakly virulent strain; the tinctorial method failed to detect coxiellae in the spleens. Mouse spleen was more sensitive for fluorescent antibody detection of Coxiella burneti than guinea pig spleen. A prerequisite of fluorescent antibody demonstration in animals is that the conjugate used should contain antibodies against the antigen in phase I.

6139

Needy, C.F. 1964. The indirect fluorescent antibody technique in scrub typhus studies. Med. J. Malaya 19:65-66.

The use of the indirect FA method in the rapid diagnosis of scrub typhus is described. The test procedure uses impression smears of mouse peritoneum and FITC-labeled antihuman globulin.

6140

Somov, G.P.; Shapiro, M.I.; Legkodimova, K.V. 1965. A study of the D. sibiricus reproduction in the human embryo tissue culture with the aid of the fluorescent antibody method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:8:39-43. In Russian.

The immunofluorescent method was applied to the study of D. sibiricus rickettsia reproduction in a monolayer of trypsinized cells of human embryo kidneys. On the basis of the data obtained, combined use of this method is considered to have future prospects for investigating the interaction of rickettsiae with the cells and rapid determination of the species of rickettsiae isolated directly on the tissue culture from various objects.

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13. ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume III contains 208 annotated literature citations, arranged according to major subject areas, and a complete author index.

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